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- KOBAYASHI, Fumie  
Tochigi 329-11 (JP)
- SHIMA, Nobuyuki  
Tochigi 329-04 (JP)
- YASUDA, Hisataka  
Kawachi-gun, Tochigi 329-04 (JP)
- NAKAGAWA, Nobuaki, Nishiura Heights 2-4  
Shimotsuga-gun, Tochigi 329-05 (JP)
- MORINAGA, Tomonori  
Tochigi 321-02 (JP)
- UEDA, Masatsugu  
Kawagoe-shi, Saitama 350-11 (JP)
- HIGASHIO, Kanji  
Saitama 350 (JP)

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(74) Representative:

Wakerley, Helen Rachael  
Reddie & Grose,  
16 Theobalds Road  
London WC1X 8PL (GB)

(71) Applicant:

SNOW BRAND MILK PRODUCTS CO., LTD.  
Sapporo-shi, Hokkaido 065 (JP)

(72) Inventors:

- GOTO, Masaaki  
Tochigi 329-05 (JP)
- TSUDA, Eisuke  
Shimotsuga-gun, Tochigi 329-05 (JP)
- MOCHIZUKI, Shin'ichi  
Tochigi 329-04 (JP)
- YANO, Kazuki-Nishiura Heights 3-1  
Tochigi 329-05 (JP)

## (54) NOVEL PROTEIN AND METHODS FOR THE PRODUCTION OF THE SAME

(57) A protein which inhibits osteoclast differentiation and/or maturation and a method of production of the protein. The protein is produced by human embryonic lung fibroblasts and has molecular weight of about 60 kD and about 120 kD under non-reducing conditions and about 60 kD under reducing conditions on SDS-polyacrylamide gel electrophoresis, respectively.

The protein can be isolated and purified from culture medium of the said fibroblasts. Furthermore, the protein can be produced by gene engineering.

The present invention includes cDNA for producing the protein by gene engineering, antibodies having specific affinity to the protein or a method for determination of the protein concentration using the antibodies.

**Description****Field of the invention**

5 This invention relates to a novel protein, osteoclastogenesis inhibitory factor (OCIF), and methods for producing the protein.

**Background of the invention**

10 Human bones are always remodelling by the repeated process of resorption and reconstitution. In the process, osteoblasts and osteoclasts are considered to be the cells mainly responsible for bone formation and bone resorption, respectively. A typical example of disease caused by the progression of abnormal bone metabolism is osteoporosis. The disease is known to be provoked by the condition in which bone resorption by osteoclasts exceeds bone formation by osteoblasts, but the mechanism of osteoporosis has not yet been completely elucidated. Osteoporosis causes pain in the bone and makes the bone fragile, leading to fracture. Since osteoporosis increases the number of bedridden old people, it has become a social issue with the increasing number of old people. Therefore, efficacious drugs for the treatment of the disease are expected to be developed. Bone mass reduction caused by the abnormal bone metabolism is thought to be prevented by inhibiting bone resorption, improving bone formation, or improving the balanced metabolism.

20 Bone formation is expected to be promoted by stimulating growth, differentiation, or activation of osteoblasts. Many cytokines are reported to stimulate growth or differentiation of osteoblasts, i.e. fibroblast growth factor (FGF) (Rodan S. B. et al., Endocrinology vol. 121, p1917, 1987), insulin-like growth factor-I (IGF-I) (Hock J.M. et al., Endocrinology vol. 122, p254, 1988), insulin-like growth factor-II (IGF-II) (McCarthy T. et al., Endocrinology vol. 124, p301, 1989), Activin A (Centrella M. et al., Mol. Cell. Biol. vol. 11, p250, 1991), Vasculotropin (Varonique M et al., Biochem. Biophys. Res. Commun. vol. 199, p380, 1994), and bone morphogenetic protein (BMP) (Yamaguchi, A et al., J. Cell Biol. vol. 113, p682, 1991, Sampath T.K. et al., J. Biol Chem. vol. 267, p20532, 1992, and Knutson R. et al., Biochem. Biophys. Res. Commun. vol. 194, p1352, 1993).

30 On the other hand, cytokines which inhibits differentiation and/or maturation of osteoclasts have been paid attention and have been intensively studied. Transforming growth factor- $\beta$  (Chenu C. et al., Proc. Natl. Acad. Sci. USA, vol. 85, p5683, 1988) and interleukin-4 (Kasano K. et al., Bone-Miner., vol. 21, p179, 1993) are found to inhibit the differentiation of osteoclasts. Calcitonin (Bone-Miner., vol. 17, p347, 1992), Macrophage colony-stimulating factor (Hattersley G. et al. J. Cell. Physiol. vol. 137, p199, 1988), interleukin-4 (Watanabe, K. et al., Biochem. Biophys. Res. Commun. vol. 172, p1035, 1990), and interferon- $\gamma$  (Gowen M. et al., J. Bone Miner. Res., vol. 1, p469, 1986) are found to inhibit bone resorption by osteoclasts.

35 These cytokines are expected to be efficacious drugs for improving bone mass reduction by stimulating bone formation and/or by inhibiting bone resorption. The cytokines such as insulin like growth factor-I and bone morphogenetic proteins are now investigated in clinical trials for their effects in treatment of patients with bone diseases. Calcitonin is already used as a drug to care osteoporosis and to diminish pain in osteoporosis.

40 Examples of drugs now clinically utilized for the treatment of bone diseases and for shortening the treatment period are dihydroxyvitamine D<sub>3</sub>, vitamin K<sub>2</sub>, calcitonin and its derivatives, hormones such as estradiol, ipriflavon, and calcium preparations. However, these drugs do not provide satisfactory therapeutic effects, and novel drug substances have been expected to be developed. As mentioned, bone metabolism is controlled in the balance between bone resorption and bone formation. Therefore, cytokines which inhibit osteoclast differentiation and/or maturation are expected to be developed as drugs for the treatment of bone diseases such as osteoporosis.

**45 Disclosure of Invention**

This invention was initiated from the view point described above. The purpose of this invention is to offer both a novel factor termed osteoclastogenesis inhibitory factor (OCIF) and a procedure to produce the factor efficiently.

50 The inventors have intensively searched for osteoclastogenesis inhibitory factors in human embryonic fibroblast IMR-90 (ATCC CCL186) conditioned medium and have found a novel osteoclastogenesis inhibitory factor (OCIF) which inhibits differentiation and/or maturation of osteoclasts.

The inventors have established a method for accumulating the protein to a high concentration by culturing IMR-90 cells using alumina ceramic pieces as the cell adherence matrices.

55 The inventors have also established an efficient method for isolating the protein, OCIF, from the IMR-90 conditioned medium using the following sequential column chromatography, ion-exchange, heparin affinity, cibacron-blue affinity, and reverse phase.

The inventors, based on the amino acid sequence of the purified natural OCIF, successfully cloned a cDNA encod-

ing this protein. The inventors established also a procedure to produce this protein which inhibits differentiation of osteoclasts. This invention concerns a protein which is produced by human lung fibroblast cells, has molecular weights in SDS-PAGE of 60 KD in the reducing conditions and 120 KD under the non-reducing conditions, has affinity for both cation-exchange resins and heparin, reduces its activity to inhibit differentiation and maturation of osteoclasts if treated for 5 10 minutes at 70 °C or for 30 minutes at 56 °C, and lose its activity to inhibit differentiation and maturation of osteoclasts by the treatment for 10 minutes at 90 °C. The amino acid sequence of the protein OCIF which is described in the present invention is clearly different from any of known factors inhibiting formation of osteoclasts.

The invention includes a method to purify OCIF protein, comprising : (1) culturing human fibroblasts, (2) applying the conditioned medium to a heparin column to obtain the adsorbed fraction, (3) purifying the OCIF protein using a cation-exchange column, (4) purifying the OCIF protein using a heparin affinity column, (5) purifying the OCIF protein using a cibacron blue affinity column, (6) isolating the OCIF protein using reverse-phase column chromatography. Cibacron blue F3GA coupled to a carrier made of synthetic hydrophilic polymers is an example of materials used to prepare Cibacron blue columns. These columns are conventionally called "blue columns".

The invention includes a method for accumulating the OCIF protein to a high concentration by culturing human fibroblasts using alumina ceramic pieces as the cell-adherence matrices.

Moreover, the inventors determined the amino acid sequences of the peptides derived from OCIF, designed the primers based on these amino acid sequences, and obtained cDNA fragments encoding OCIF from a cDNA library of IMR-90 cells.

## 20 Detailed description of the invention

The OCIF protein of the present invention can be isolated from human fibroblast conditioned medium with high yield. The procedure to isolate OCIF is based on ordinary techniques for purifying proteins from biomaterials, in accordance with the physical and chemical properties of OCIF protein. For example, concentrating procedure includes ordinary biochemical techniques such as ultrafiltration, lyophilization, and dialysis. Purifying procedure includes combinations of several chromatographic techniques for purifying proteins such as ion-exchange column chromatography, affinity column chromatography, gel filtration column chromatography, hydrophobic column chromatography, reverse phase column chromatography, and preparative gel electrophoresis. The human fibroblast used for production of the OCIF protein is preferably IMR-90. A method for producing the IMR-90 conditioned medium is preferably a process comprising, adhering human embryonic fibroblast IMR-90 cells to alumina ceramic pieces in roller-bottles, using DMEM medium supplemented with 5 % new born calf serum for the cell culture, and cultivating the cells in roller-bottles for 7 to 10 days by stand cultivation. CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) is preferably added to the buffer as a detergent in the purification steps of OCIF protein.

OCIF protein of the instant invention can be initially obtained as a heparin binding basic OCIF fraction by applying 35 the culture medium to a heparin column (Heparin-Sepharose CL-6B, Pharmacia), eluting with 10 mM Tris-HCl buffer, pH 7.5, containing 2 M NaCl, and then by applying the OCIF fraction to a Q + anion-exchange column (HiLoad-Q/FF, Pharmacia), and collecting non-adsorbed fraction. OCIF protein can be purified by subjecting the obtained OCIF fraction to purification on a S + cation-exchange column (HiLoad-S/FF, Pharmacia), a heparin column (Heparin-5PW, TOSOH), Cibacron Blue column (Blue-5PW, TOSOH), and a reverse-phase column (BU-300 C4, Perkin Elmer) and 40 the material is defined by the previously described properties.

The present invention relates to a method of cloning cDNA encoding the OCIF protein based on the amino acid sequence of natural OCIF and a method of obtaining recombinant OCIF protein that inhibits differentiation and/or maturation of osteoclasts. The OCIF protein is purified according to the method described in the present invention and is treated with endopeptidase (for example, lysylendopeptidase). The amino acid sequences of the peptides produced by 45 the digestion are determined and the mixture of oligonucleotides that can encode each internal amino acid sequence was synthesized. The OCIF cDNA fragment is obtained by PCR (preferably RT-PCR, reverse transcriptase PCR) using the oligonucleotide mixtures described above as primers. The full length OCIF cDNA encoding the OCIF protein is cloned from a cDNA library using the obtained OCIF DNA fragment as a probe. The OCIF cDNA containing the entire coding region is inserted into an expression vector. The recombinant OCIF can be produced by expressing the OCIF 50 cDNA containing the entire coding region in mammalian cells or bacteria.

The present invention relates to the novel proteins OCIF2, OCIF3, OCIF4, and OCIF5 that are variants of OCIF and have the activity described above. These OCIF variants are obtained from the cDNA library constructed with IMR-90 poly(A) + RNA by hybridization using the OCIF cDNA fragment as a probe. Each of the OCIF variant cDNAs containing the entire coding region is inserted into an expression vector. Each recombinant OCIF variant can be produced by 55 expressing each of the OCIF variant cDNAs containing the entire coding region in the conventional hosts. Each recombinant OCIF variant can be purified according to the method described in this invention. Each recombinant OCIF variant has an ability to inhibit osteoclastogenesis.

The present invention further includes OCIF mutants. They are substitution mutants comprising replacement of one

5      lane 15 ; molecular weight marker proteins  
      lane 16 ; a monomer type nOCIF protein  
      lane 17 ; a dimer type nOCIF protein  
      lane 18 ; a monomer type rOCIF(E) protein  
      lane 19 ; a dimer type rOCIF(E) protein  
      lane 20 ; a monomer type rOCIF(C) protein  
      lane 21 ; a dimer type rOCIF(C) protein

10     Figure 9 shows comparison of amino acid sequences between OCIF and OCIF2.  
      Figure 10 shows comparison of amino acid sequences between OCIF and OCIF3.  
      Figure 11 shows comparison of amino acid sequences between OCIF and OCIF4.  
      Figure 12 shows comparison of amino acid sequences between OCIF and OCIF5.  
      Figure 13 shows standard curve for determination of OCIF protein concentration by an EIA employing anti-OCIF polyclonal antibodies.  
15     Figure 14 shows standard curve for determination of OCIF protein concentration by an EIA employing anti-OCIF monoclonal antibodies.  
      Figure 15 shows the effect of rOCIF protein on osteoporosis.

20     Best Mode for Carrying Out the Invention

25     The present invention will be further explained by the following examples, however, the scope of the invention is not restricted to the examples.

30     EXAMPLE 1

35     Preparation of a conditioned medium of human fibroblast IMR-90

40     Human fetal lung fibroblast IMR-90 (ATCC-CCL186) cells were cultured on alumina ceramic pieces (80 g) (alumina: 99.5%, manufactured by Toshiba Ceramic K.K.) in DMEM medium (manufactured by Gibco BRL Co.) supplemented with 5% CS and 10mM HEPES buffer (500 ml/roller bottle) at 37°C under the presence of 5% CO<sub>2</sub> for 7 to 10 days using 60 roller bottles (490 cm<sup>2</sup>, 110 x 171mm, manufactured by Coning Co.) in static culture. The conditioned medium was harvested, and a fresh medium was added to the roller bottles. About 30L of IMR-90 conditioned medium per batch culture was obtained. The conditioned medium was designated as sample 1.

45     EXAMPLE 2

50     Assay method for osteoclast development inhibitory activity

55     Osteoclast development inhibitory activity was assayed by measuring tartrate-resistant acid phosphatase(TRAP) activity according to the methods of M. Kumegawa et.al (Protein - Nucleic Acid • Enzyme, vol.34 p999, 1989) and N. Takahashi et.al (Endocrinology, vol.122, p1373, 1988) with modifications. Briefly, bone marrow cells obtained from 17 day-old mouse were suspended in α-MEM (manufactured by GIBCO BRL Co.) containing 10% FBS, 2x10<sup>-8</sup> M of activated vitamin D<sub>3</sub>, and each test sample, and were inoculated to each well of 96-well plate at a cell density of 3x10<sup>5</sup> cells/0.2 ml/well. The plates were incubated for 7 days at 37°C in humidified 5%CO<sub>2</sub>. Cultures were further continued by replacing 0.16 ml of old medium with the same volume of fresh medium on day 3 and day 5 after starting cultivation. On day 7, after washing the plates with phosphate buffered saline, cells were fixed with ethanol/acetone (1:1) for 1 min. at room temperature, and then osteoclast development was tested by determining for phosphatase activity using a kit (Acid Phosphatase, Leucocyte, Catalog No. 387-A, manufactured by Sigma Co.). The decrease of TRAP positive cells was taken as an indication of OCIF activity.

60     EXAMPLE 3

65     Purification of OCIF

70     i) Heparin Sepharose CL-6B column chromatography

75     The 90L of IMR-90 conditioned medium (sample 1) was filtrated with 0.22 μ membrane filter (hydrophilic Milidisk, 2000 cm<sup>2</sup>, Milipore Co.), and was divided into three portions. Each portion (30 l) was applied to a heparin Sepharose

CL-6B column (5 x 4.1 cm, Pharmacia Co.) equilibrated with 10mM Tris-HCl containing 0.3M NaCl, pH 7.5. After washing the column with 10mM Tris-HCl, pH 7.5 at a flow rate of 500 ml/hr., heparin Sepharose CL-6B adsorbent protein fraction was eluted with 10mM Tris-HCl, pH 7.5, containing 2M NaCl. The fraction was designated as sample 2.

5 ii) HiLoad-Q/FF column chromatography

The heparin Sepharose-adsorbent fraction (sample 2) was dialyzed against 10mM Tris-HCl, pH 7.5, supplemented with CHAPS to a final concentration of 0.1%, incubated at 4 °C overnight, and divided into two portions. Each portion was then applied to an anion-exchange column (HiLoad-Q/FF, 2.6 x 10 cm, Pharmacia Co.) which was equilibrated with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5 to obtain a non-adsorbent fraction (1000 ml). The fraction was designated as sample 3.

iii) HiLoad-S/HP column chromatography

15 The HiLoad-Q non-adsorbent fraction (sample 3) was applied to a cation-exchange column (HiLoad-S/HP, 2.6 x 10 cm, Pharmacia Co.) which was equilibrated with 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with linear gradient from 0 to 1 M NaCl at a flow rate of 8 ml/min for 100 min. and fractions (12 ml) were collected. Each ten fractions from number 1 to 40 was pooled to form one portion. Each 100 µl of the four portions was tested for OCIF activity. OCIF activity was observed in 20 fractions from 11 to 30 (as shown in Figure 1). The fractions from 21 to 30 which had higher specific activity were collected and was designated as sample 4.

iv) Heparin-5PW affinity column chromatography

25 One hundred and twenty ml of HiLoad-S fraction from 21 to 30 (sample 4) was diluted with 240 ml of 50 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to heparin-5PW affinity column (0.8 x 7.5 cm, Tosoh Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with linear gradient from 0 to 2M NaCl at a flow rate of 0.5ml/min for 60 min. and fractions (0.5 ml) were collected. Fifty µl was removed from each fraction to test for OCIF activity. The active fractions, eluted with 0.7 to 1.3M NaCl was pooled and was designated as sample 5.

v) Blue 5PW affinity column chromatography

35 Ten ml of sample 5 was diluted with 190 ml of 50mM Tris-HCl, 0.1% CHAPS, pH 7.5 and applied to a blue-5PW affinity column, (0.5x5 cm, Tosoh Co.) which was equilibrated with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 50mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted with a 30 ml linear gradient from 0 to 2M NaCl at a flow rate of 0.5 ml/min., and fractions (0.5 ml) were collected. Using 25 µl of each fraction, OCIF activity was evaluated. The fractions number 49 to 70, eluted with 1.0-1.6M NaCl had OCIF activity.

40 vi) Reverse phase column chromatography

45 The blue 5PW fraction obtained by collecting fractions from 49 to 50 was acidified with 10µl of 25% TFA and applied to a reverse phase C4 column (BU-300, 2.1x220mm, manufactured by Perkin-Elmer) which was equilibrated with 0.1% of TFA and 25% of acetonitrile. The adsorbed protein was eluted with linear gradient from 25 to 55% acetonitrile at a flow rate of 0.2 ml/min. for 60 min., and each protein peak was collected (Fig.3). One hundred µl of each peak fraction was tested for OCIF activity, and peak 6 and the peak 7 had OCIF activity. The result was shown in Table 1.

Table 1

OCIF activity eluted from reverse phase C4 column				
Sample	Dilution			
	1/40	1/120	1/360	1/1080
Peak 6	++	++	+	-
Peak 7	++	+	-	-

[ ++ means OCIF activity inhibiting osteoclast development more than 80%, + means OCIF activity inhibiting osteoclast development between 30% and 80%, and - means no OCIF activity.]

## EXAMPLE 4

## Molecular weight of OCIF protein

The two protein peaks (6 and 7) with OCIF activity were subjected to SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions. Briefly, 20 $\mu$ l of each peak fraction was concentrated under vacuum and dissolved in 1.5 $\mu$ l of 10mM Tris-HCl, pH 8, 1mM EDTA, 2.5% SDS, 0.01% bromophenol blue, and incubated at 37°C overnight under non-reducing conditions or under reducing conditions (with 5% of 2-mercaptoethanol). Each 1.0  $\mu$ l of sample was then analyzed by SDS-polyacrylamide gel electrophoresis with a gradient gel of 10-15% acrylamide (Pharmacia Co.) and an electrophoresis-device (Fast System, Pharmacia Co.). The following molecular weight marker proteins were used to calculate molecular weight : phosphorylase b (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.0 kD), and lactalbumin (14.4 kD). After electrophoresis, protein bands were visualized by silver stain using Phast Silver Stain Kit. The results were shown in Fig. 4.

A protein band with an apparent 60 KD was detected in the peak 6 protein under both reducing and non-reducing conditions. A protein band with an apparent 60 KD was detected under reducing conditions and a protein band with an apparent 120 KD was detected under non-reducing conditions in the peak 7 protein. Therefore, the protein of peak 7 was considered to be a homodimer of the protein of peak 6.

## EXAMPLE 5

## Thermostability of OCIF

Twenty  $\mu$ l of sample from the blue-5PW fractions 51 and 52 was diluted to 30 $\mu$ l with 10 mM phosphate buffered saline, pH 7.2, and incubated for 10 min. at 70°C or 90 °C, or for 30 min. at 56°C. The heat-treated samples were tested for OCIF activity. The results were shown in Table 2.

Table 2

Thermostability of OCIF			
Sample	Dilution		
	1/300	1/900	1/2700
untreated	++	+	-
70°C, 10 min	+	-	-
56°C, 30 min	+	-	-
90°C, 10 min	-	-	-

[ ++ means OCIF activity inhibiting osteoclast development more than 80%, + means OCIF activity inhibiting osteoclast development between 30% and 80%, and - means no OCIF activity.]

## EXAMPLE 6

Internal amino acid sequence of OCIF prot in

5 Each 2 fractions (1 ml) from No. 51-70 of blue-5PW fraction was acidified with 10  $\mu$ l of 25% TFA, and was applied to a reverse phase C4 column (BU-300, 2.1x220mm, manufactured by Perkin-Elmer Co.) equilibrated with 25% of acetonitrile containing 0.1 % TFA. The adsorbed protein was eluted with a 12 ml linear gradient of 25 to 55% acetonitrile at a flow rate of 0.2 ml/min, and the protein fractions corresponding to peak 6 and peak 7 were collected, respectively. The protein of each peak was applied to a protein sequencer (PROCISE 494, Perkin-Elmer Co.). However, the N-terminal sequence of the protein of each peak could not be analyzed. Therefore, N-terminal of the protein of each peak was considered to be blocked. So, internal amino acid sequences of these proteins were analyzed.

10 The protein of peak 6 or peak 7 purified by C4-HPLC was concentrated by centrifugation and pyridil-ethylated under reducing conditions. Briefly, 50  $\mu$ l of 0.5 M Tris-HCl, pH 8.5, containing 100  $\mu$ g of dithiothreitol, 10 mM EDTA, 7 M guanidine-HCl, and 1% CHAPS was added to each samples, and the mixture was incubated overnight in the dark at a room 15 temperature. Each the mixture was acidified with 25% TFA (a final concentration 0.1%) and was applied to a reversed phase C4 column (BU-300, 2.1x30mm, Perkin-Elmer Co.) equilibrated with 20 % acetonitrile containing 0.1 % TFA. The pyridil-ethylated OCIF protein was eluted with a 9 ml linear gradient from 20 to 50% acetonitrile at a flow rate of 0.3 ml/min, and each protein peak was collected. The pyridil-ethylated OCIF protein was concentrated under vacuum, and dissolved in 25  $\mu$ l of 0.1 M Tris-HCl, pH 9, containing 8 M Urea, and 0.1 % Tween 80. Seventy three  $\mu$ l of 0.1 M Tris-HCl, pH 9, and 0.02  $\mu$ g of lysyl endopeptidase (Wako Pure Chemical, Japan) were added to the tube, and incubated at 37 °C for 15 hours. Each digest was acidified with 1  $\mu$ l of 25% TFA and was applied to a reverse phase C8 column (RP-20, 2.1x220mm, Perkin-Elmer Co.) equilibrated with 0.1% TFA. 20 The peptide fragments were eluted from the column with linear gradient from 0 to 50 % acetonitrile at a flow rate of 0.2 ml/min for 70 min., and each peptide peak was collected. Each peptide fragment (P1 - P3) was applied to the protein 25 sequencer. The sequences of the peptides were shown in Sequence Numbers 1 - 3, respectively.

## EXAMPLE 7

Determination of nucleotide sequence of the OCIF cDNA

30 i) Isolation of poly(A) + RNA from IMR-90 cells

About 10  $\mu$ g of poly(A) + RNA was isolated from  $1 \times 10^8$  cells of IMR-90 by using Fast Track mRNA isolation kit (Invitrogen) according to the manufacturer's instructions.

35 ii) Preparation of mixed primers

The following two mixed primers were synthesized based on the amino acid sequences of two peptides (peptide P2 and peptide P3, sequence numbers 2 and 3, respectively). All the oligonucleotides in the mixed primers No. 2F can 40 code for the amino acid sequence from the sixth residue, glutamine (Gln) to the twelfth residue, leucine (Leu), in peptide P2. All the oligonucleotides in the mixed primers No. 3R can code for the amino acid sequence from the sixth residue, histidine (His), to the twelfth residue, lysine (Lys), in peptide P3. The sequences of the mixed primers No. 2F and No. 3R were shown in Table 3.

45

50

55

Table 3

5

No. 2F

10 5' -CAAGAACAAA CTTTCAATT-3'  
 G G G C C GC  
 A  
 15 G

20

No. 3R

25 5' -TTTATACATT GTAAAAGAAT G-3'  
 C G C G GCTG  
 A C  
 30 G T

## 35 iii) Amplification of OCIF cDNA fragment by PCR (Polymerase chain reaction)

First strand cDNA was generated using Superscript II cDNA synthesis kit (Gibco BRL) and 1 ug of poly (A) + RNA obtained in the example 7-i) according to the manufacturer's instructions. The DNA fragment encoding OCIF was obtained by PCR using the cDNA template and the primers shown in EXAMPLE 7-ii).

40 PCR was performed with the conditions as follows;

45

10X Ex Taq Buffer (Takara Shuzo)	5 ul
2.5 mM solution of dNTPs	4 ul
cDNA solution	1 ul
Ex Taq (Takara Shuzo)	0.25 ul
sterile distilled water	29.75 ul
40 uM solution of primers No. 2F	5 ul
40 uM solution of primers No. 3R	5 ul

50

55 The components of the reaction were mixed in a microcentrifuge tube. An initial denaturation step at 95 °C for 3 min was followed by 30 cycles of denaturation at 95°C for 30 sec annealing at 50 °C for 30 sec and extention at 70 °C for 2min. After the amplification, final extention step was performed at 70 °C for 5min. The size of PCR products were determined on a 1.5 % agarose gel electrophoresis. About 400 bp OCIF DNA fragment was obtained.

## EXAMPLE 8

## Cloning of the OCIF cDNA fragment amplified by PCR and determination of its DNA sequence

5 The OCIF cDNA fragment amplified by PCR in EXAMPLE 7-iii) was inserted in the plasmid, pBluescript II SK<sup>+</sup> using DNA ligation kit ver. 2 (Takara Shuzo) according to the method by Marchuk, D. et al. (Nucleic Acids Res., vol 19, p1154, 1991). E.coli. DH5  $\alpha$  (Gibco BRL) was transformed with ligation mixture. The transformants were grown and a plasmid containing the OCIF cDNA (about 400 bp) was purified using the commonly used method. This plasmid was called pBSOCIF. The sequence of OCIF cDNA in pBSOCIF was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The size of the OCIF cDNA is 397 bp. The OCIF cDNA encodes an amino acid sequence containing 132 residues. The amino acid sequences of the internal peptides (peptide P2 and peptide P3, sequence number 2 and 3, respectively) that were used to design the primers were found at N- or C- terminal side in the amino acid sequence of the 132 amino acid polypeptide predicted by the 397 bp OCIF cDNA. In addition, the amino acid sequence of the internal peptide P1 (sequence number 1) was also found in the predicted amino acid sequence of the polypeptide. These data show that the 397 bp OCIF cDNA is a portion of the full length OCIF cDNA.

## EXAMPLE 9

## Preparation of the DNA probe

20 The 397 bp OCIF cDNA was prepared according to the conditions described in EXAMPLE 7-iii). The OCIF cDNA was subjected to a preparative agarose gel electrophoresis. The OCIF cDNA was purified from the gel using QIAEX gel extraction kit (QIAGEN), labeled with [ $\alpha$ <sup>32</sup>P]dCTP using Megaprime DNA labeling system (Amersham) and used to select a phage containing the full length OCIF cDNA.

## EXAMPLE 10

## Preparation of the cDNA library

30 cDNA was generated using Great Lengths cDNA synthesis kit (Clontech), oligo (dT) primer, [ $\alpha$ <sup>32</sup>P]dCTP and 2.5  $\mu$ g of poly(A) + RNA obtained in the example 7-i) according to the manufacturer's instructions. EcoRI-Sall-NotI adaptor was ligated to the cDNA. The cDNA was separated from the free adaptor and unincorporated free [ $\alpha$ <sup>32</sup>P]dCTP. The purified cDNA was precipitated with ethanol and dissolved in 10  $\mu$ l of TE buffer (10 mM Tris-HCl (pH8.0), 1 mM EDTA). The cDNA with the adaptor was inserted in  $\lambda$ ZAP EXPRESS vector (Stratagene) at EcoRI site. The recombinant  $\lambda$ ZAP EXPRESS phage DNA containing the cDNA was in vitro packaged using Gigapack gold II packaging extract (Stratagene) and recombinant  $\lambda$ ZAP EXPRESS phage library was prepared.

## EXAMPLE 11

## 40 Screening of recombinant phage

Recombinant phages obtained in EXAMPLE 10 were infected to E. Coli, XL1-Blue MRF' (Stratagene) at 37 °C for 15 min.. The infected E.coli cells were added to NZY medium containing 0.7 % agar at 50°C and plated on the NZY agar plates. After the plates were incubated at 37 °C overnight, Hybond N (Amersham) were placed on the surface of plates containing plaques. The membranes were denatured in the alkali solution, neutralized, and washed in 2xSSC according to the standard protocol. The phage DNA was immobilized on the membranes using UV Crosslink (Stratagene). The membranes were incubated in the hybridization buffer (Amersham) containing 100  $\mu$ g/ml salmon sperm DNA at 65°C for 4 hours and then incubated at 65 °C overnight in the same buffer containing  $2 \times 10^5$  cpm/ml denatured OCIF DNA probe. The membranes were washed twice with 2xSSC and twice with a solution containing 0.1xSSC and 0.1 % SDS at 65 °C for 10 min each time. The positive clones were purified by repeating the screening twice. The purified  $\lambda$ ZAP EXPRESS phage clone containing about 1.6 kb DNA insert was used in the experiments described below. This phage was called  $\lambda$ OCIF. The purified  $\lambda$ OCIF and the infected into E. Coli XL1-Blue MRF' (Stratagene) according to a protocol of  $\lambda$ ZAP EXPRESS cloning kit (Stratagene). The culture broth of infected XL1-Blue MRF' was prepared. Purified 1OCIF and ExAssist helper phage (Stratagene) were co-infected into E. coli strain XL-1 blue MRF' according to the protocol supplied with the kit. The culture broth of the co-infected XL-1 blue MRF' was added to a culture of E. coli strain XLOR (Stratagene) to transform them. Thus we obtained a Kanamycin-resistant transformant harboring a plasmid designated pBKOCIF which is a pBKCMV (Stratagene) vector containing the 1.6 kb insert fragment. The transformant including the plasmid containing about 1.6 kb OCIF cDNA was obtained by picking up the kanamycin-

5 resistant colonies. The plasmid was called pBKOCIF. The transformant has been deposited to National Institute of Bio-science and Human-Technology (NIBH), Agency of Industrial Science and Technology as "FERM BP-5267" as pBK/O1F10. A national deposit (Accession number, FERM P-14998) was transferred to the international deposit, on October 25, 1995 according to the Budapest treaty. The transformant pBK/O1F10 was grown and the plasmid pBKOCIF was purified according to the standard protocol.

## EXAMPLE 12

10 Determination of the nucleotide sequence of OCIF cDNA containing the full coding region.

15 The nucleotide sequence of OCIF cDNA obtained in EXAMPLE 11 was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The primers used were T3, T7 primers (Stratagene) and synthetic primers designed according to the OCIF cDNA sequence. The sequences of these primers are shown in sequence numbers 16 to 29. The nucleotide sequence of the OCIF cDNA is shown in sequence number 6 and the amino acid sequence predicted by the cDNA sequence is shown in sequence number 5.

## EXAMPLE 13

20 Production of recombinant OCIF by 293/EBNA cells

## i) Construction of the plasmid for expressing OCIF cDNA

25 pBKOCIF containing about 1.6 kb OCIF cDNA was prepared as described in EXAMPLE 11, and digested with restriction enzymes, BamHI and Xhol. The OCIF cDNA insert was cut out, separated by an agarose gel electrophoresis, and purified using QIAEX gel extraction kit (QIAGEN). The purified OCIF cDNA insert was ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) digested with restriction enzymes, BamHI and Xhol. E.coli. DH5 $\alpha$  (Gibco BRL) was transformed with the ligation mixture. The transformants were grown and the plasmid containing the OCIF cDNA (about 1.6 kb) was purified using QIAGEN column (QIAGEN). The expression plasmid pCEPOCIF was precipitated with ethanol, and dissolved in sterile distilled water was used in the experiments described below.

## ii) Transient expression of OCIF cDNA and analysis of the biological activity

30 Recombinant OCIF was produced using the expression plasmid, pCEPOCIF prepared in EXAMPLE 13-i) according to the method described below.  $8 \times 10^5$  cells of 293/EBNA (Invitrogen) were inoculated in each well of the 6-well plate using IMDM containing 10 % fetal calf serum (Gibco BRL). After the cells were incubated for 24 hours, the culture medium was removed and the cells were washed with serum free IMDM. The expression plasmid, pCEPOCIF and lipofectamine (Gibco BRL) were diluted with OPTI-MEM (Gibco BRL) and were mixed, and added to the cells in each well according to the manufacturer's instructions. Three  $\mu$ g of pCEPOCIF and 12  $\mu$ l of lipofectamine were used for each transfection. After the cells were incubated with pCEPOCIF and lipofectamine for 38 hours, the medium was replaced with 1 ml of OPTI-MEM. After the transfected cells were incubated for 30 hours, the conditioned medium was harvested and used for the biological assay. The biological activity of OCIF was analysed according to the method described below. Bone marrow cells obtained from mice, 17 days-old, were suspended in  $\alpha$ -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS,  $2 \times 10^{-8}$ M activated vitamin D<sub>3</sub>, and each test sample, and were inoculated and cultured for 7 days at 37°C in humidified 5%CO<sub>2</sub> as described in EXAMPLE 2. During incubation, 160  $\mu$ l of old medium in each well was replaced with the same volume of the fresh medium containing test sample diluted with 1 $\times$ 10 $^{-8}$ M of activated vitamin D<sub>3</sub> and  $\alpha$ -MEM containing FBS on day 3 and day 5. On day 7, after washing the wells with phosphate buffered saline, cells were fixed with ethanol/acetone (1:1) for 1 min. and then osteoclast development was tested using acid phosphatase activity measuring kit (Acid Phosphatase, Leucocyte, Catalog No. 387-A, Sigma Co.). The decrease of the number of TRAP positive cells was taken as an OCIF activity. As result, the conditioned medium showed the same OCIF activity as natural OCIF protein from IMR-90 conditioned medium (Table 4).

Table 4

OCIF activity of 293/EBNA conditioned medium.							
Cultured Cell	Dilution						
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
OCIF expression vector transfected	++	++	++	++	++	+	-
vector transfected	-	-	-	-	-	-	-
untreated	-	-	-	-	-	-	-

[ ++ ; OCIF activity inhibiting osteoclast development more than 80%, + ; OCIF activity inhibiting osteoclast development between 30% and 80%, and - ; no OCIF activity. ]

15

iii) Isolation of recombinant OCIF protein from 293/EBNA-conditioned medium

20 293/EBNA-conditioned medium (1.8 l) obtained by cultivating the cells described in example 13-ii) was supplemented with 0.1 % of CHAPS and filtrated with 0.22  $\mu$ m membrane filter (Stereobics GS, Milipore Co.). The conditioned medium was applied to 50 ml of a heparin Sepharose CL-6B column (2.6 x 10 cm, Pharmacia Co.) equilibrated with 10mM Tris-HCl, pH 7.5. After washing the column with 10mM Tris-HCl, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 4 ml/min for 100 min. and fractions (8 ml) were collected. Using 150  $\mu$ l of each fraction, OCIF activity was assayed according to the method described in EXAMPLE 2.

25 OCIF active fraction (112 ml) eluted with approximately 0.6 to 1.2 M NaCl was obtained.

One hundred twelve ml of the active fraction was diluted to 1000 ml with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to a heparin affinity column (heparin-5PW, 0.8 x 7.5 cm, Tosoh Co.) equilibrated with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 10mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 0.5ml/min for 60 min., and fractions (0.5 ml) were collected. Four  $\mu$ l of each fraction was analyzed by SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions as described in EXAMPLE 4. On SDS-PAGE under reducing conditions, a single band of rOCIF protein with an apparent 60 KD was detected in fractions from 30 to 32, under non-reducing conditions, bands of rOCIF protein with an apparent 60 KD and 120 KD were also detected in fractions from 30 to 32. The isolated rOCIF fraction from 30 to 32 was designated as recombinant OCIF derived from 293/EBNA (rOCIF(E)). 1.5 ml of the rOCIF(E) (535  $\mu$ g/ml) was obtained when determined by the method of Lowry using bovine serum albumin as a standard protein.

#### EXAMPLE 14

##### Production of recombinant OCIF using CHO cells

40 i) Construction of the plasmid for expressing OCIF

pBKOCIF containing about 1.6 kb OCIF cDNA was prepared as described in EXAMPLE 11, and digested with restriction enzymes, Sall and EcoRV. About 1.4 kb OCIF cDNA insert was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The expression vector, pcDL-SR  $\alpha$ 296 (Molecular and Cellular Biology, vol 8, p466, 1988) was digested with restriction enzymes, PstI and KpnI. About 3.4 kb of the expression vector fragment was cut out, separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The ends of the purified OCIF cDNA insert and the expression vector fragment were blunted using DNA blunting kit (Takara Shuzo). The purified OCIF cDNA insert and the expression vector fragment were ligated using DNA ligation kit ver. 2 (Takara Shuzo). E.coli. DH5a  $\alpha$  (Gibco BRL) was transformed with the ligation mixture. The transformant containing the OCIF expression plasmid, pSR $\alpha$ OCIF was obtained.

55 ii) Preparation of expression plasmid

The transformant containing the OCIF expression plasmid, pSR  $\alpha$ OCIF prepared in the example 13-i) and the transformant containing the mouse DHFR expression plasmid, pBAdDSV shown in WO92/01053 were grown according to the standard method. Both plasmids were purified by alkali treatment, polyethylene glycol precipitation, and cesium chrolide density gradient ultra centrifugation according to method of Maniatis et al. (Molecular cloning, 2nd edition).

## iii) Adaptation of CHOdhFr- cells to the protein free medium

5 CHOdhFr- cells (ATCC, CRL 9096) were cultured in IMDM containing 10 % fetal calf serum. The cells were adapted to EX-CELL 301 (JRH Bioscience) and then adapted to EX-CELL PF CHO (JRH Bioscience) according to the manufacturer's instructions.

## iv) Transfection of the OCIF expression plasmid, and the mouse DHFR expression plasmid, to CHOdhFr- cells.

CHOdhFr- cells prepared in EXAMPLE 14-iii) were transfected by electroporation with pSRαOCIF and pBAdDSV

10 prepared in EXAMPLE 14-ii). 200 µg of pSRαOCIF and 20 µg of pBAdDSV were dissolved under sterile conditions in 0.8 ml of IMDM (Gibco BRL) containing 10 % fetal calf serum CG.  $2 \times 10^7$  cells of CHOdhFr- were suspended in 0.8 ml of this medium. The cell suspension was transferred to a cuvette (Bio Rad) and the cells were transfected by electroporation using gene pulser (Bio Rad) under condition of 360 V and 960 µF. The suspension of electroporated cells was transferred to T-flasks (Sumitomo Bakelite) containing 10 ml of EX-CELL PF-CHO, and incubated in the CO<sub>2</sub> incubator

15 for 2 days. Then the transfected cells were inoculated in each well of a 96 well plate (Sumitomo Bakelite) at a density of 5000 cells/well and cultured for about 2 weeks. The transformants expressing DHFR are selected since EX-CELL PF-CHO does not contain nucleotides and the parental cell line CHO dhFr- can not grow in this medium. Most of the transformants expressing DHFR express OCIF since the OCIF expression plasmid was used ten times as much as the mouse DHFR expression plasmid. The transformants whose conditioned medium had high OCIF activity were selected

20 among the transformants expressing DHFR according to the method described in EXAMPLE 2. The transformants that express large amounts of OCIF were cloned by limiting dilution. The clones whose conditioned medium had high OCIF activity were selected as described above and the transformant expressing large amount of OCIF, 5561, was obtained.

## v) Production of recombinant OCIF

25 To produce recombinant OCIF (rOCIF), EX-CELL 301 medium (3 l) in a 3 l-spiner flask was inoculated with the clone (5561) at a cell-density of  $1 \times 10^5$  cells/ml. The 5561 cells were cultured in a spinner flask at 37°C for 4 to 5 days. When the concentration of the 5561 cells reached to  $1 \times 10^6$  cells/ml, about 2.7 l of the conditioned medium was harvested. Then about 2.7 l of EX-CELL 301 was added to the spinner flask and the 5561 cells were cultured repeatedly.

30 About 20 l of the conditioned medium was harvested using the three spinner flasks.

## vi) Isolation of recombinant OCIF protein from CHO cells-conditioned medium

35 CHOcells-conditioned medium (1.0 l) described in EXAMPLE 14-v) was supplemented with 1.0 g of CHAPS and filtered with 0.22 µm membrane filter (Sterebecks GS, Millipore Co.). The conditioned medium was applied to a heparin Sepharose-FF column (2.6 x 10 cm, Pharmacia Co.) equilibrated with 10 mM Tris-HCl, pH 7.5. After washing the column with 10 mM Tris-HCl, 0.1 % CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 2 M NaCl at a flow rate of 4 ml/min for 100 min. and fractions (8 ml) were collected. Using 150 µl of each fraction, OCIF activity was assayed according to the method described in EXAMPLE 2. Active fraction (112 ml) eluted with

40 approximately 0.6 to 1.2 M NaCl was obtained.

The 112 ml of active fraction was diluted to 1200 ml with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, and applied to a affinity column (blue-5PW, 0.5 x 5.0 cm, Tosoh Co.) equilibrated with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5. After washing the column with 10 mM Tris-HCl, 0.1% CHAPS, pH 7.5, the adsorbed protein was eluted from the column with linear gradient from 0 to 3 M NaCl at a flow rate of 0.5 ml/min for 60 min., and fractions (0.5 ml) were collected. Four µl of each fraction was subjected to SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions as described in EXAMPLE 4. On SDS-PAGE under reducing conditions, a single band of rOCIF protein with apparent 60 KD was detected in fractions 30 to 38, under non-reducing conditions, bands of rOCIF protein with apparent 60 KD and 120 KD were also detected in fractions 30 to 38. The isolated rOCIF fraction, 30 to 38, was designated as purified recombinant OCIF derived from CHO cells (rOCIF(C)). 4.5 ml of the rOCIF(C) (113 µg/ml) was obtained when determined by the method of Lowry using bovine serum albumin as a standard protein.

## EXAMPLE 15

## Determination of N-terminal amino acid sequence of rOCIFs

55 Each 3 µg of the isolated rOCIF(E) and rOCIF(C) was adsorbed to polyvinylidene difluoride (PVDF) membranes with Prospin (PERKIN ELMER Co.). The membranes were washed with 20 % ethanol and the N-terminal amino acid sequences of the adsorbed proteins were analyzed by protein sequencer (PROCISE 492, PERKIN ELMER Co.). The

determined N-terminal amino acid sequence is shown in sequence No. 7.

The N-terminal amino acid of rOCIF(E) and rOCIF(C) was the 22th amino acid of glutamine from Met as translation starting point, as shown in sequence number 5. The 21 amino acids from Met to Gin were identified as a signal peptide. The N-terminal amino acid sequence of OCIF isolated from IMR-90 conditioned medium was undetectable. Accordingly, the N-terminal glutamine of OCIF may be blocked by converting from glutamine to pyroglutamine within culturing or purifying.

#### EXAMPLE 16

##### 10 Biological activity of recombinant(r) OCIF and natural(n) OCIF

###### i) Inhibition of vitamin D<sub>3</sub> induced osteoclast formation from murine bone marrow cells

Each the rOCIF(E) and nOCIF sample was diluted with  $\alpha$ -MEM (GIBCO BRL Co.) containing 10% FBS and  $2 \times 10^{-8}$  M of activated vitamin D<sub>3</sub> (a final concentration of 250 ng/ml). Each sample was serially diluted with the same medium, and 100  $\mu$ l of each diluted sample was added to each well in 96-well plates. Bone marrow cells obtained from mice, 17 days-old, were inoculated at a cell density of  $3 \times 10^5$  cells/100  $\mu$ l/well to each well in 96-well plates and cultured for 7 days at 37°C in humidified 5%CO<sub>2</sub>. On day 7, the cells were fixed and stained with a acid phosphatase measuring kit (Acid Phosphatase, Leucocyte, No387-A, Sigma) according to the method described in EXAMPLE 2. The decrease of acid phosphatase activity (TRAP) was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated by solubilizing the pigment of dye and measuring absorbance. In detail, 100  $\mu$ l of a mixture of 0.1 N NaOH and dimethylsulfoxide (1:1) was added to each well and the well was vibrated to solubilize the dye. After solubilizing the dye completely, an absorbance of each well was measured at 590 nm subtracting the absorbance at 490 nm using microplate reader (Immunoreader NJ-2000, InterMed). The microplate reader was adjusted to 0 absorbance using a well with monolayered bone marrow cells which was cultured in the medium without activated vitamin D<sub>3</sub>. The decrease of TRAP activity was expressed as a percentage of the control absorbance value (=100%) of the solubilized dye from wells with bone marrow cells which were cultured in the absence of OCIF. The results are shown in Table 5.

Table 5

Inhibition of vitamin D <sub>3</sub> -induced osteoclast formation from murine bone marrow cells						
OCIF concentration(ng/ml)	250	125	63	31	16	0
rOCIF(E)	0	0	3	62	80	100
nOCIF	0	0	27	27	75	100 (%)

40 Both nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 16 ng/ml or higher

###### ii) Inhibition of vitamin D<sub>3</sub>-induced osteoclast formation in co-cultures of stromal cells and mouse spleen cells.

45 Effect of OCIF on osteoclast formation induced by Vitamin D<sub>3</sub> in co-cultures of stromal cells and mouse spleen cells was tested according to the method of N. Udagawa et al. (Endocrinology, vol. 125, p1805-1813, 1989). In detail, each of rOCIF(E), rOCIF(C), and nOCIF sample was serially diluted with  $\alpha$ -MEM (GIBCO BRL Co.) containing 10% FBS,  $2 \times 10^{-8}$  M of activated vitamin D<sub>3</sub>, and  $2 \times 10^{-7}$  M dexamethasone, and 100  $\mu$ l of each of the diluted samples was added to each well in 96 well-microwell plates. Murine bone marrow-derived stromal ST2 cells (RIKEN Cell Bank RCB0224) ;  $5 \times 10^3$  cells per 100  $\mu$ l of  $\alpha$ -MEM containing 10% FBS, and spleen cells from ddY mice, 8 weeks-old, ;  $1 \times 10^5$  cells per 100  $\mu$ l in the same medium, were inoculated to each well in 96-well plates and cultured for 5 days at 37°C in humidified 5%CO<sub>2</sub>. On day 5, the cells were fixed and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma). The decrease of acid phosphatase-positive cells was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated according to the method described in EXAMPLE 16-i). The results are shown in Table 6 ; rOCIF(E) and rOCIF(C), and Table 7 ; rOCIF(E) and nOCIF.

Table 6

Inhibition of osteoclast formation in co-cultures of stromal cells and mouse spleen cells.					
OCIF concentration(ng/ml)	50	25	13	6	0
rOCIF(E)	3	22	83	80	100
rOCIF(C)	13	19	70	96	100 (%)

Table 7

Inhibition of osteoclast formation in co-cultures of stromal cells and mouse spleen cells.				
OCIF concentration(ng/ml)	250	63	16	0
rOCIF(E)	7	27	37	100
rOCIF(C)	13	23	40	100 (%)

nOCIF, rOCIF(E) and rOCIF(C) inhibited osteoclast formation in a dose dependent manner in the concentration of 6 - 16 ng/ml or higher

## iii) Inhibition of PTH-induced osteoclast formation from murine bone marrow cells.

Effect of OCIF on osteoclast formation induced by PTH was tested according to the method of N. Takahashi et al. (Endocrinology, vol. 122, p1373-1382, 1988). In detail, each the rOCIF(E) and nOCIF sample (125 ng/ml) was serially diluted with  $\alpha$ -MEM (manufactured by GIBCO BRL Co.) containing 10% FBS and  $2 \times 10^{-8}$ M PTH, and 100 $\mu$ l of each the diluted samples was added to 96 well-plates. Bone marrow cells from ddY mice, 17 days-old, at a cell density of  $3 \times 10^5$  cells per 100 $\mu$ l of  $\alpha$ -MEM containing 10% FBS were inoculated to each well in 96-wells plates and cultured for 5 days at 37°C in humidified 5%CO<sub>2</sub>. On day 5, the cells were fixed with ethanol/aceton (1:1) for 1 min. at room temperature and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma) according to the method described in EXAMPLE 2. The decrease of acid phosphatase-positive cells was taken as OCIF activity. The decrease of acid phosphatase-positive cells was evaluated according to the method described in EXAMPLE 16-i). The results are shown in Table 8.

Table 8

Inhibition of PTH-induced osteoclast formation from murine bone marrow cells.						
OCIF concentration(ng/ml)	125	63	31	16	8	0
rOCIF(E)	6	58	58	53	88	100
nOCIF	18	47	53	56	91	100

nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 16 ng/ml or higher

## iv) Inhibition of IL-11-induced osteoclast formation

Effect of OCIF on osteoclast formation induced by IL-11 was tested according to the method of T. Tamura et al. (Proc. Natl. Acad. Sci. USA, vol. 90, p11924-11928, 1993). In detail, each rOCIF(E) and nOCIF sample was serially

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5 diluted with  $\alpha$ -MEM (GIBCO BRL Co.) containing 10% FBS and 20 ng/ml IL-11 and 100 $\mu$ l of each the diluted sample was added to each well in 96-well plates. Newborn mouse calvaria-derived pre-adipocyte MC3T3-G2/PA6 cells (RIKEN Cell Bank RCB1127) ; 5x10<sup>3</sup> cells per 100 $\mu$ l of  $\alpha$ -MEM containing 10% FBS, and spleen cells from ddY mouse, 8 weeks-old, ; 1x10<sup>5</sup> cells per 100  $\mu$ l in the same medium, were inoculated to each well in 96-well plates and cultured for 5 days at 37 °C in humidified 5%CO<sub>2</sub>. On day 5, the cells were fixed and stained with a kit for acid phosphatase (Acid Phosphatase, Leucocyte, No387-A, Sigma). Acid phosphatase positive cells were counted under microscope and a decrease of the cell numbers was taken as OCIF activity. The results are shown in Table 9.

10 Table 9

OCIF concentration(ng/ml)	500	125	31	7.8	2.0	0.5	0
nOCIF	0	0	1	4	13	49	31
rOCIF(E)	0	0	1	3	10	37	31

15 Both nOCIF and rOCIF(E) inhibited osteoclast formation in a dose dependent manner in the concentration of 2 ng/ml or higher

20 The results shown in Table 4-8 indicated that OCIF inhibits all the vitamin D<sub>3</sub>, PTH, and IL-11-induced osteoclast formations at almost the same doses. Accordingly, OCIF would be able to be used for treatment of the different types of bone disorders with decreased bone mass, which are caused by different substances which induce bone resorption.

25 EXAMPLE 17

Isolation of monomer-type OCIF and dimer-type OCIF

30 Each rOCIF(E) and rOCIF(C) sample containing 100  $\mu$ g of OCIF protein, was supplemented with 1/100 volume of 25 % trifluoro acetic acid and applied to a reverse phase column (PROTEIN-RP, 2.0x250 mm, YMC Co.) equilibrated with 30 % acetonitrile containing 0.1 % trifluoro acetic acid. OCIF protein was eluted from the column with linear gradient from 30 to 55 % acetonitrile at a flow rate of 0.2 ml/min for 50 min. and each OCIF peak was collected. Each the monomer-type OCIF peak fraction and dimer-type OCIF peak fraction was lyophilized, respectively.

35 EXAMPLE 18

Determination of molecular weight of recombinant OCIFs

40 Each 1  $\mu$ g of the isolated monomer-type and dimer-type nOCIF purified using reverse phase column according to EXAMPLE 3-iv) and each 1  $\mu$ g of monomer-type and dimer-type rOCIF described in EXAMPLE 17 was concentrated under vaccum, respectively. Each sample was incubated in the buffer for SDS-PAGE, subjected to SDS-polyacrylamide gel electrophoresis, and protein bands on the gel were stained with silver according to the method described in EXAMPLE 4. Results of electrophoresis under non-reducing conditions and reducing conditions are shown in Figure 6 and Figure 7.

45 A protein band with an apparent molecular weight of 60 KD was detected in each monomer-type OCIF sample, and a protein band with an apparent molecular weight of 120 KD was detected in each dimer-type OCIF sample in non-reducing conditions. A protein band with an apparent molecular weight of 60 KD was detected in each monomer-type OCIF sample under reducing conditions. Accordingly, molecular weights of monomer-type nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells and rOCIF from CHO cells were almost the same. Molecular weights of dimer-type nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells, and rOCIF from CHO cells were also the same.

50 EXAMPLE 19

Remove N-linked Oligosaccharide chain and Mesuring molecular weight of natural and recombinant OCIF

55 Each sample containing 5 $\mu$ g of the isolated monomer-type and dimer-type nOCIF purified using reverse phase column according to EXAMPLE 3-iv) and each sample containing 5  $\mu$ g of monomer-type and dimer-type rOCIF described in EXAMPLE 17 were concentrated under vaccum. Each sample was dissolved in 9.5  $\mu$ l of 50 mM sodium phosphate buffer, pH 8.6, containing 100 mM 2-mercaptoethanol, supplemented with 0. 5  $\mu$ l of 250 U/ml N-glycanase (Seikagaku

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kogyo Co.) and incubated for one day at 37 °C. Each sample was supplemented with 10 µl of 20 mM Tris-HCl, pH 8.0 containing 2 mM EDTA, 5 % SDS, and 0.02 % bromo-phenol blue and heated for 5 min at 100 °C. Each 1 µl of the samples was subjected to SDS-polyacrylamide gel electrophoresis, and protein bands on the gel were stained with silver as described in EXAMPLE 4. The patterns of electrophoresis are shown in Figure 8.

5 An apparent molecular weight of each the deglycosylated nOCIF from IMR-90 cells, rOCIF from CHO cells, and rOCIF from 293/EBNA cells was 40 KD under reducing conditions. An apparent molecular weight of each untreated nOCIF from IMR-90 cells, rOCIF from 293/EBNA cells, and rOCIF from CHO cells was 60 KD under reducing conditions. Accordingly, the results indicate that the OCIF proteins are glycoproteins with N-linked sugar chains.

### 10 EXAMPLE 20

#### Cloning of OCIF variant cDNAs and determination of their DNA sequences

15 The plasmid pBKOCIF, which is inserted OCIF cDNA to pBKCMV (Stratagene), was obtained from one of some purified positive phage as in example 10 and 11. And more, during the screening of the cDNA library with the 397 bp OCIF cDNA probe, the transformants containing plasmids whose insert sizes were different from that of pBKOCIF were obtained. These transformants containing the plasmids were grown and the plasmids were purified according to the standard method. The sequence of the insert DNA in each plasmid was determined using Taq Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer). The used primers were T3, T7 primers (Stratagene) and synthetic primers prepared based on the nucleotide sequence of OCIF cDNA. There are four OCIF variants (OCIF2, 3, 4, and 5) in addition to OCIF. The nucleotide sequence of OCIF2 is shown in the sequence number 8 and the amino acid sequence of OCIF2 predicted by the nucleotide sequence is shown in the sequence number 9. The nucleotide sequence of OCIF3 is shown in the sequence number 10 and the amino acid sequence of OCIF3 predicted by the nucleotide sequence is shown in the sequence number 11. The nucleotide sequence of OCIF4 is shown in the sequence number 12 and the amino acid sequence of OCIF4 predicted by the nucleotide sequence is shown in the sequence number 13. The nucleotide sequence of OCIF5 is shown in the sequence number 14 and the amino acid sequence of OCIF5 predicted by the nucleotide sequence is shown in the sequence number 15. The structures of OCIF variants are shown in Figures 9 to 12 and are described in brief below. OCIF2

20 OCIF2 cDNA has a deletion of 21 bp from guanine at nucleotide number 265 to guanine at nucleotide number 285 in OCIF cDNA (sequence number 6). Accordingly OCIF2 has a deletion of 7 amino acids from glutamic acid (Glu) at amino acid number 68 to glutamine (Gln) at amino acid number 74 in OCIF (sequence number 5).

25 OCIF3

30 OCIF3 cDNA has a point mutation at nucleotide number 9 in OCIF cDNA (sequence number 6) where cytidine is replaced with guanine. Accordingly OCIF3 has a mutation and asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys). The mutation seems to be located in the signal sequence and have no essential effect on the secreted OCIF3. OCIF3 cDNA has a deletion of 117 bp from guanine at nucleotide number 872 to cytidine at nucleotide number 988 in OCIF cDNA (sequence number 6). Accordingly OCIF3 has a deletion of 39 amino acids from threonine (Thr) at amino acid number 270 to leucine (Leu) at amino acid number 308 in OCIF (sequence number 5).

#### OCIF4

40 OCIF4 cDNA has two point mutations in OCIF cDNA (sequence number 6). Cytidine at nucleotide number 9 is replaced with guanine and guanine at nucleotide number 22 is replaced with thymidine in OCIF cDNA (sequence number 6). Accordingly OCIF4 has two mutations. Asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys), and alanine (Ala) at amino acid number -14 is replaced with serine (Ser). These mutations seem to be located in the signal sequence and have no essential effect on the secreted OCIF4. OCIF4 cDNA has about 4 kb DNA, which is the intron 2 of OCIF gene, inserted between nucleotide number 400 and nucleotide number 401 in OCIF cDNA (sequence number 6). The open reading frame stops in intron 2. Accordingly OCIF4 has an additional novel amino acid sequence containing 21 amino acids after alanine (Ala) at amino acid number 112 in OCIF (sequence number 5).

## OCIF5

OCIF5 cDNA has a point mutation at nucleotide number 9 in OCIF cDNA (sequence number 6) where cytidine is replaced with guanine.

5 Accordingly OCIF5 has a mutation and asparagine (Asn) at amino acid number -19 in OCIF (sequence number 5) is replaced with lysine (Lys). The mutation seems to be located in the signal sequence and have no essential effect on the secreted OCIF5.

OCIF5 cDNA has the latter portion (about 1.8 kb) of intron 2 between nucleotide number 400 and nucleotide number 401 in OCIF cDNA (sequence number 6). The open reading frame stops in the latter portion of intron 2.

10 Accordingly OCIF5 has an additional novel amino acid sequence containing 12 amino acids after alanine (Ala) at amino acid number 112 in OCIF (sequence number 5).

## EXAMPLE 21

## 15 Production of OCIF variants

## i) Construction of the plasmid for expressing OCIF variants

The plasmid containing OCIF2 or OCIF3 cDNA was obtained as described in EXAMPLE 20 and called pBKOCIF2 and pBKOCIF3, respectively. pBKOCIF2 and pBKOCIF3 were digested with restriction enzymes, BamHI and Xhol. The OCIF2 and OCIF3 cDNA inserts were separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified OCIF2 and OCIF3 cDNA inserts were individually ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) that had been digested with restriction enzymes, BamHI and Xhol. E. coli. DH5 $\alpha$  (Gibco BRL) was transformed with the ligation mixture.

25 The plasmid containing OCIF4 cDNA was obtained as described in EXAMPLE 20 and called pBKOCIF4. pBKOCIF4 was digested with restriction enzymes, SpeI and Xhol (Takara Shuzo). The OCIF4 cDNA insert was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified OCIF4 cDNA insert was ligated using DNA ligation kit ver. 2 (Takara Shuzo) to the expression vector pCEP4 (Invitrogen) that had been digested with restriction enzymes, NheI and Xhol (Takara Shuzo). E.coli. DH5  $\alpha$  (Gibco BRL) was transformed with the ligation mixture.

30 The plasmid containing OCIF5 cDNA was obtained as described in EXAMPLE 20 and was called pBKOCIF5. pBKOCIF5 was digested with restriction enzyme, HindIII (Takara Shuzo). The 5' portion of the coding region in the OCIF5 cDNA insert was separated by agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The OCIF expression plasmid, pCEPOCIF, obtained in EXAMPLE 13-i) was digested with restriction enzyme, HindIII (Takara Shuzo). The 5' portion of the coding region in the OCIF cDNA was removed. The rest of the plasmid that contains pCEP vector and the 3' portion of the coding region of OCIF cDNA was called pCEPOCIF-3'. pCEPOCIF-3' was separated by an agarose gel electrophoresis, and purified from the gel using QIAEX gel extraction kit (QIAGEN). The OCIF5 cDNA HindIII fragment and pCEPOCIF-3' were ligated using DNA ligation kit ver. 2 (Takara Shuzo). E.coli. DH5  $\alpha$  (Gibco BRL) was transformed with the ligation mixture.

40 The obtained transformants were grown at 37 °C overnight and the OCIF variants expression plasmids (pCEPOCIF2, pCEPOCIF3, pCEPOCIF4, and pCEPOCIF5) were purified using QIAGEN column (QIAGEN). These OCIF-variants-expression plasmids were precipitated with ethanol, dissolved in sterile distilled water, and used in the experiments described below.

45 ii) Transient expression of OCIF variant cDNAs and analysis of the biological activity of recombinant OCIF variants.

Recombinant OCIF variants were produced using the expression plasmid, pCEPOCIF2, pCEPOCIF3, pCEPOCIF4, and pCEPOCIF5 prepared as described in EXAMPLE 21-i) according to the method described in EXAMPLE 13-ii). The biological activities of recombinant OCIF variants were analyzed. The results were that these OCIF variants (OCIF2, OCIF3, OCIF4, and OCIF5) had a weak activity.

## EXAMPLE 22

## Preparation of OCIF mutants

55 i) Construction of a plasmid vector for subcloning cDNAs encoding OCIF mutants

The plasmid vector (5  $\mu$ g) described in EXAMPLE 11 was digested with restriction enzymes Bam HI and Xho I (

Takara Shuzo). The digested DNA was subjected to a preparative agarose gel electrophoresis. DNA fragment with an approximate size of 1.6 kilobase pairs (kb) that contained the entire coding sequence for OCIF was purified from the gel using QIAEX gel extraction kit (QIAGEN). The purified DNA was dissolved in 20  $\mu$ l of sterile distilled water. This solution was designated DNA solution 1. p Bluescript II SK + (3  $\mu$ g) (Stratagene) was digested with restriction enzymes Bam HI and Xho I (Takara Shuzo). The digested DNA was subjected to preparative agarose gel electrophoresis. DNA fragment with an approximate size of 3.0 kb was purified from the gel using QIAEX DNA extraction kit (QIAGEN). The purified DNA was dissolved in 20  $\mu$ l of sterile distilled water. The solution was designated DNA solution 2. One microliter of DNA solution 2, 4  $\mu$ l of DNA solution 1 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 (Takara Shuzo) were mixed and incubated at 16 °C for 30 min. (The ligation mixture was used for the transformation of *E. coli* in a manner described below). Conditions for transformation of *E. coli* were as follows. One hundred microliters of competent *E. coli* DH5  $\alpha$  cells (GIBCO BRL) and 5  $\mu$ l of the ligation mixture was mixed in a sterile 15-ml tube (IWAKI glass). The tube was kept on ice for 30 min. After incubation for 45 sec at 42°C, to the cells was added 250  $\mu$ l of L broth (1% Tryptone, 0.5% yeast extract, 1% NaCl). The cell suspension was then incubated for 1 hr. at 37°C with shaking. Fifty microliters of the cell suspension was plated onto an L-agar plate containing 50  $\mu$ g/ml of ampicillin. The plate was incubated overnight at 37°C.

15 Six colonies which grew on the plate were individually incubated in 2 ml each of L-broth containing 50  $\mu$ g/ml of ampicillin overnight at 37°C with shaking. The structure of the plasmids in the colonies was analyzed. A plasmid in which the 1.6-kb DNA fragment containing the entire OCIF cDNA is inserted between the digestion sites of Bam HI and Xho I of pBluescript II SK + was obtained and designated as pSK + -OCIF.

20 ii) Preparation of mutants in which one of the Cys residues in OCIF is replaced with Ser residue

1) Introduction of mutations into OCIF cDNA

OCIF mutants were prepared in which one of the five Cys residues present in OCIF at positions 174, 181, 256, 298 and 379 (in SEQUENCE NO 4) was replaced with Ser residue and were designated OCIF-C19S (174Cys to Ser), OCIF-C20S (181Cys to Ser), OCIF-C21S (256Cys to Ser), OCIF-C22S (298Cys to Ser) and OCIF-C23S (379Cys to Ser), respectively.

25 To prepare the mutants, nucleotides encoding the corresponding Cys residues were replaced with those encoding Ser. Mutagenesis was carried out by a two-step polymerase chain reaction (PCR). The first step of the PCRs consisted of two reactions, PCR 1 and PCR 2.

35	PCR 1	10X Ex Taq Buffer (Takara Shuzo)	10 $\mu$ l
		2.5 mM solution of dNTPs	8 $\mu$ l
		the plasmid vector described in EXAMPLE 11 (8ng/ml)	2 $\mu$ l
		sterile distilled water	73.5 $\mu$ l
		20 $\mu$ M solution of primer 1	5 $\mu$ l
		100 $\mu$ M solution of primer 2 (for mutagenesis)	1 $\mu$ l
		Ex Taq (Takara Shuzo)	0.5 $\mu$ l
	PCR 2	10X Ex Taq Buffer (Takara Shuzo)	10 $\mu$ l
		2.5 mM solution of dNTPs	8 $\mu$ l
		the plasmid vector described in EXAMPLE 11 (8ng/ml)	2 $\mu$ l
40		sterile distilled water	73.5 $\mu$ l
		20 $\mu$ M solution of primer 3	5 $\mu$ l
		100 $\mu$ M solution of primer 4 (for mutagenesis)	1 $\mu$ l
		Ex Taq (Takara Shuzo)	0.5 $\mu$ l
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55 Specific sets of primers were used for each mutation and other components were unchanged. Primers used for the reactions are shown in Table 10. The nucleotide sequences of the primers are shown in SEQUENCE NO: 20,23,27 and 30-40. The PCRs were performed under the following conditions as follows. An initial denaturation step at 97°C for 3 min was followed by 25 cycles of denaturation at 95°C for 1 min annealing at 55°C for 1 min and extension at 72°C for

3 min. After these amplification cycles, final extension was performed at 70°C for 5 min. The size of the PCR products was confirmed by agarose gel electrophoresis using reaction solution. After the first PCR, excess primers were removed using Amicon microcon (Amicon). The final volume of the solutions that contained the PCR products were made to 50 µl with sterile distilled water. These purified PCR products were used for the second PCR (PCR 3).

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PCR 3	10X Ex Taq Buffer (Takara Shuzo)	10 µl
	2.5 mM solution of dNTPs	8 µl
	solution containing DNA fragment obtained from PCR 1	5 µl
	solution containing DNA fragment obtained from PCR 2	5 µl
	sterile distilled water	61.5 µl
15	20 µM solution of primer 1	5 µl
	20 µM solution of primer 3	5 µl
	Ex Taq (Takara Shuzo)	0.5 µl

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Table 10

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mutants	primer-1	primer-2	primer-3	primer-4
OCIF-C19S	IF 10	C19SR	IF 3	C19SF
OCIF-C20S	IF 10	C20SR	IF 3	C20SF
OCIF-C21S	IF 10	C21SR	IF 3	C21SF
OCIF-C22S	IF 10	C22SR	IF 14	C22SF
OCIF-C23S	IF 6	C23SR	IF 14	C23SF

The reaction conditions were exactly the same as those for PCR 1 or PCR 2. The size of the PCR products was confirmed by 1.0 % or 1.5 % agarose gel electrophoresis. The DNA fragments were precipitated with ethanol, dried under vacuum and dissolved in 40 µl of sterile distilled water. The solutions containing DNA fragments with mutation C19S, C20S, C21S, C22S and C23S were designated as DNA solution A, DNA solution B, DNA solution C, DNA solution D and DNA solution E, respectively.

The DNA fragment which is contained in solution A (20 µl) was digested with restriction enzymes Nde I and Sph I (Takara Shuzo). A DNA fragment with an approximate size of 400 base pairs (bp) was extracted from a preparative agarose gel and dissolved in 20 µl of sterile distilled water. This DNA solution was designated DNA solution 3. Two micrograms of pSK + -OCIF was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 4.2 kb was purified from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 µl of sterile distilled water. This DNA solution was designated as DNA solution 4. Two microliters of DNA solution 3, 3 µl of DNA solution 4 and 5 µl of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5 µl of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C19S.

The DNA fragment which is contained in solution B (20 µl) was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 µl of sterile distilled water. This DNA solution was designated DNA solution 5. Two microliters of DNA solution 5, 3 µl of DNA solution 4 and 5 µl of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5 µl of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C20S. The DNA fragment which is contained in solution C (20 µl) was digested with restriction enzymes Nde I and Sph I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 µl of sterile distilled water. This DNA solution was designated as DNA solution 6. Two micro-

liters of DNA solution 6, 3  $\mu$ l of DNA solution 4 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C21S.

5 The DNA fragment which is contained in solution D (20  $\mu$ l) was digested with restriction enzymes Nde I and Bst PI. A DNA fragment with an approximate size of 600 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 7. Two micrograms of pSK + -OCIF was digested with restriction enzymes Nde I and Bst PI. A DNA fragment with an approximate size of 4.0 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 8. Two microliters of DNA solution 7, 3  $\mu$ l of DNA solution 8 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA in which the 600-bp Nde I-Bst PI fragment with the mutation (the C22S mutation) is substituted for the 600-bp Nde I-Bst PI fragment of pSK+ -OCIF by analyzing the DNA structure. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C22S.

10 The DNA fragment which is contained in solution E (20  $\mu$ l) was digested with restriction enzymes Bst PI and Eco RV. A DNA fragment with an approximate size of 120 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 9. Two micrograms of pSK + -OCIF was digested with restriction enzymes Bst EII and Eco RV. A DNA fragment with an approximate size of 4.5 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 10. Two microliters of DNA solution 9, 3  $\mu$ l of DNA solution 10 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened 15 for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-C23S.

## 2) Construction of vectors for expressing the OCIF mutants

20 pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S and pSK-OCIF-C23S were digested with restriction enzymes Bam HI and Xho I. The 1.6 kb Bam HI-Xho I DNA fragment encoding each OCIF mutant was isolated and dissolved in 20  $\mu$ l of sterile distilled water. The DNA solutions that contain 1.6 kb cDNA fragments derived from pSK-OCIF-C19S, pSK-OCIF-C20S, pSK-OCIF-C21S, pSK-OCIF-C22S and pSK-OCIF-C23S were designated C19S DNA solution, C20S DNA solution, C21S DNA solution, C22S DNA solution and C23S DNA solution, respectively. Five 25 micrograms of a expression vector pCEP 4 (Invitrogen) was digested with restriction enzymes Bam HI and Xho I. A DNA fragment with an approximate size of 10 kb was purified and dissolved in 40  $\mu$ l of sterile distilled water. This DNA solution was designated as pCEP 4 DNA solution. One microliter of pCEP 4 DNA solution and 6  $\mu$ l of either C19SDNA solution, C20S DNA solution, C21S DNA solution, C22S DNA solution or C23S DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent *E. coli* 30 DH5 $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmid in which a 1.6-kb cDNA fragment is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmide which were obtained containing the cDNA encoding OCIF-C19S, OCIF-C20S, OCIF-C21S, OCIF-C22S and OCIF-C23S were designated pCEP4-OCIF-C19S, pCEP4-OCIF-C20S, pCEP4-OCIF-C21S, pCEP4-OCIF-C22S and pCEP4-OCIF-C23S, respectively.

35 ii) Preparation of domain-deletion mutants of OCIF

### (1) deletion mutagenesis of OCIF cDNA

40 A series of OCIF mutants with deletions of from Thr 2 to Ala 42, from Pro 43 to Cys 84, from Glu 85 to Lys 122, from Arg 123 to Cys 164, from Asp 177 to Gin 251 and from Ile 252 to His 326 were prepared (positions of the amino acid residues are shown in SEQUENCE NO: 4). These mutants were designated as OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2, respectively.

45 Mutagenesis was performed by two-step PCR as described in EXAMPLE 22-(ii). The primer sets for the reactions are shown in Table 11 and the nucleotide sequences of the primers are shown in SEQUENCE NO: 19, 25, 40-53, and 54.

Table 11

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-DCR1	Xhol F	DCR1R	IF 2	DCR1F
OCIF-DCR2	Xhol F	DCR2R	IF 2	DCR2F
OCIF-DCR3	Xhol F	DCR3R	IF 2	DCR3F
OCIF-DCR4	Xhol F	DCR4R	IF 16	DCR4F
OCIF-DDD1	IF 8	DDD1R	IF 14	DDD1F
OCIF-DDD2	IF 8	DDD2R	IF 14	DDD2F

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The final PCR products were precipitated with ethanol, dried under vacuum and dissolved in 40 $\mu$ l of sterile distilled water. Solutions of DNA fragment coding for portions of OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2 were designated as DNA solutions F, G, H, I, J and K, respectively.

The DNA fragment which is contained in solution F (20  $\mu$ l) was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated DNA solution 11. Two micrograms of pSK+ -OCIF was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 4.0 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated DNA solution 12. Two microliters of DNA solution 11, 3  $\mu$ l of DNA solution 12 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR1.

The DNA fragment which is contained in solution G (20  $\mu$ l) was digested with restriction enzymes Nde I and Xho I.

A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 13. Two microliters of DNA solution 13, 3  $\mu$ l of DNA solution 12 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation was carried out. Competent E. coli DH5a cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR2.

The DNA fragment which is contained in solution H (20  $\mu$ l) was digested with restriction enzymes Nde I and Xho I. A DNA fragment with an approximate size of 500 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 14. Two microliters of DNA solution 14, 3  $\mu$ l of DNA solution 12 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR3.

The DNA fragment which is contained in solution I (20  $\mu$ l) was digested with restriction enzymes Xho I and Sph I. A DNA fragment with an approximate size of 900 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 15. Two micrograms of pSK+ -OCIF was digested with restriction enzymes Xho I and Sph I. A DNA fragment with an approximate size of 3.6 kb was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20 $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 16. Two microliters of DNA solution 15, 3  $\mu$ l of DNA solution 16 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DCR4.

The DNA fragment which is contained in solution J (20  $\mu$ l) was digested with restriction enzymes BstP I and Nde I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 17. Two microliters of DNA solution 17, 3  $\mu$ l of DNA solution 8 and 5 $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent E. coli DH5  $\alpha$  cells were transformed with 5 $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by

restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DDD1. The DNA fragment which is contained in solution K (20  $\mu$ l) was digested with restriction enzymes Nde I and BstP I. A DNA fragment with an approximate size of 400 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 18. Two microliters of DNA solution 18, 3  $\mu$ l of DNA solution 8 and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-DDD2.

10 2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-DCR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1 and pSK-OCIF-DDD2 were digested with restriction enzymes Bam HI and Xho I. The Bam HI-Xho I DNA fragment containing entire coding sequence for each OCIF mutant was isolated and dissolved in 20  $\mu$ l of sterile distilled water. These DNA solutions that contain the Bam HI-Xho I fragment derived from pSK-OCIF-DCR1, pSK-OCIF-DCR2, pSK-OCIF-DCR3, pSK-OCIF-DCR4, pSK-OCIF-DDD1 and pSK-OCIF-DDD2 were designated DCR1 DNA solution, DCR2 DNA solution, DCR3 DNA solution, DCR4 DNA solution, DDD1 DNA solution and DDD2 DNA solution, respectively. One microliter of pCEP 4 DNA solution and 6  $\mu$ l of either DCR1 DNA solution, DCR2 DNA solution, DCR3 DNA solution, DCR4 DNA solution, DDD1 DNA solution or DDD2 DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent *E. coli* DH5  $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA in which the DNA fragment with deletions is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids containing the cDNA encoding OCIF-DCR1, OCIF-DCR2, OCIF-DCR3, OCIF-DCR4, OCIF-DDD1 and OCIF-DDD2 were designated as pCEP4-OCIF-DCR1, pCEP4-OCIF-DCR2, pCEP4-OCIF-DCR3, pCEP4-OCIF-DCR4, pCEP4-OCIF-DDD1 and pCEP4-OCIF-DDD2, respectively.

iii) Preparation of OCIF with C-terminal domain truncation

(1) mutagenesis of OCIF cDNA

30 A series of OCIF mutants with deletions of from Cys at amino acid residue 379 to Leu 380, from Ser 331 to Leu 380, from Asp 252 to Leu 380, from Asp 177 to Leu 380, from Arg 123 to Leu 380 and from Cys 86 to Leu 380 was prepared. Positions of the amino acid residues are shown in SEQUENCE NO: 4. These mutants were designated as OCIF-CL, OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3, respectively.

35 Mutagenesis for OCIF-CL was performed by the two-step PCR as described in EXAMPLE 22-(ii). The primer set for the reaction is shown in Table 12. The nucleotide sequences of the primers are shown in SEQUENCE NO:23, 40, 55, and 56. The final PCR products were precipitated with ethanol, dried under vacuum and dissolved in 40  $\mu$ l of sterile distilled water. This DNA solution was designated as solution L.

40 The DNA fragment which is contained in solution L (20  $\mu$ l) was digested with restriction enzymes BstP I and EcoR V. A DNA fragment with an approximate size of 100 bp was extracted from a preparative agarose gel with QIAEX gel extraction kit and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as DNA solution 19. Two microliters of DNA solution 19, 3  $\mu$ l of DNA solution 10 (described in EXAMPLE 22-(ii)) and 5  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were mixed and ligation reaction was carried out. Competent *E. coli* DH5  $\alpha$  cells were transformed with 5  $\mu$ l of the ligation mixture. Ampicillin-resistant transformants were screened for a clone containing a plasmid DNA. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmid thus obtained was named pSK-OCIF-CL. Mutagenesis of OCIF cDNA to prepare OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3 was performed by a one-step PCR.

45 PCR reactions for mutagenesis to prepare OCIF-CC, OCIF-CDD2, OCIF-CDD1, OCIF-CCR4 and OCIF-CCR3

5	10X Ex Taq Buffer (Takara Shuzo)	10 $\mu$ l
	2.5 mM solution of dNTPs	8 $\mu$ l
	the plasmid vector containing the entire OCIF cDNA described in EXAMPLE 11 (8ng/ml)	2 $\mu$ l
	sterile distilled water	73.5 $\mu$ l
10	20 $\mu$ M solution of primer OCIF Xho F	5 $\mu$ l
	100 $\mu$ M solution of primer (for mutagenesis)	1 $\mu$ l
	Ex Taq (Takara Shuzo)	0.5 $\mu$ l

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Table 12

mutants	primer-1	primer-2	primer-3	primer-4
OCIF-CL	IF 6	CL R	IF 14	CL F

Specific primers were used for each mutagenesis and other components were unchanged. Primers used for the mutagenesis are shown in Table 13. Their nucleotide sequences are shown in SEQUENCE NO:57-61. The components of each PCR were mixed in a microcentrifuge tube and PCR was performed as follows. The microcentrifuge tubes were treated for 3 minutes at 97 °C and then incubated sequentially, for 30 seconds at 95 °C, 30 seconds at 50 °C and 3 minutes at 70 °C. This three-step incubation procedure was repeated 25 times, and after that, the tubes were incubated for 5 minutes at 70 °C. An aliquot of the reaction mixture was removed from each tube and analyzed by an agarose gel electrophoresis to confirm the size of each product.

The size of the PCR products was confirmed on an agarose gel. Excess primers in the PCRs were removed using Amicon microcon (Amicon) after completion of the reaction. The DNA fragments were precipitated with ethanol, dried under vacuum and dissolved in 40  $\mu$ l of sterile distilled water. The DNA fragment in each DNA solution was digested with restriction enzymes Xho I and Bam HI. After the reactions, DNA was precipitated with ethanol, dried under vacuum and dissolved in 20  $\mu$ l of sterile distilled water.

The solutions containing DNA fragment with the CC deletion, the CDD2 deletion, the CDD1 deletion, the CCR4 deletion and the CCR3 deletion were designated as CC DNA solution, CDD2 DNA solution, CDD1 DNA solution, CCR4 DNA solution and CC R3 DNA solution, respectively.

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Table 13

mutants	primers for the mutagenesis
OCIF-CC	CC R
OCIF-CDD2	CDD2 R
OCIF-CDD1	CDD1 R
OCIF-CCR4	CCR4 R
OCIF-CCR3	CCR3 R

45

## (2) Construction of vectors for expressing the OCIF mutants

pSK-OCIF-CL was digested with restriction enzymes Bam HI and Xho I. The Bam HI-Xho I DNA fragment containing the entire coding sequence for OCIF-CL was isolated and dissolved in 20  $\mu$ l of sterile distilled water. This DNA solution was designated as CL DNA solution. One microliter of pCEP 4 DNA solution and 6  $\mu$ l of either of CL DNA solution, CC DNA solution, CDD2 DNA solution, CDD1 DNA solution, CCR4 DNA solution or CCR3 DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent

E. coli DH5 $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmids which have the desirable mutations in OCIF cDNA by analyzing the DNA structure. In each plasmid, OCIF cDNA fragment having a deletion were inserted between the recognition sites of Xho I and Bam HI of pCEP 4. The plasmids containing the cDNA encoding OCIF-CL, OCIF-CC, OCIF-CDD1, OCIF-CDD2, OCIF-CCR4 and OCIF-CCR3 were designated pCEP4-OCIF-CL, pCEP4-OCIF-CC, pCEP4-OCIF-CDD2, pCEP4-OCIF-CDD1, pCEP4-OCIF-CCR4 and pCEP4-OCIF-CCR3, respectively.

5 iv) Preparation of OCIF mutants with C-terminal truncation

10 (1) Introduction of C-terminal truncation to OCIF

A series of OCIF mutants with C-terminal truncation was prepared. OCIF mutant in which 10 residues of from Gln at 371 to Leu at 380 are replaced with 2 residues of Leu-Val was designated OCIF-CBst. OCIF mutant in which 83 residues of from Cys 298 to Leu 380 are replaced with 3 residues of Ser-Leu-Asp was designated OCIF-CSph. OCIF mutant in which 214 residues of from Asn 167 to Leu 380 are removed was designated OCIF-CBsp. OCIF mutant in which 319 residues of from Asp 62 to Leu 380 are replaced with 2 residues of Leu-Val was designated OCIF-CPst. Positions of the amino acid residues are shown in SEQUENCE NO: 4.

Two micrograms each of pSK + -OCIF was digested with one of the restriction enzymes, Bst PI, Sph I, Pst I (Takara Shuzo), and Bsp EI (New England Biolabs), and followed by phenol extraction and ethanol precipitation. The precipitated DNA was dissolved in 10  $\mu$ l of sterile distilled water. Ends of the DNAs in 2  $\mu$ l of each solution were blunted using a DNA blunting kit in final volumes of 5  $\mu$ l. To the reaction mixtures, 1  $\mu$ g (1  $\mu$ l) of an Amber codon-containing Xba I linker (5'-CTAGTCTAGACTAG-3') and 6  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 were added.

20 After the ligation reactions, 6  $\mu$ l each of the reaction mixtures was used to transform E. coli DH5 $\alpha$ . Ampicillin-resistant transformants were screened for clones containing plasmids. DNA structure was analyzed by restriction enzyme mapping and by DNA sequencing. The plasmids thus obtained were named pSK-OCIF-CBst, pSK-OCIF-CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst, respectively.

25 (2) Construction of vectors for expressing the OCIF mutants

30 pSK-OCIF-CBst, pSK-OCIF- CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst were digested with restriction enzymes Bam HI and Xho I. The 1.5 kb of DNA fragment containing entire coding sequence for each OCIF mutant was isolated and dissolved in 20  $\mu$ l of sterile distilled water. These DNA solutions that contain the Bam HI-Xhol fragment derived from pSK-OCIF-CBst, pSK-OCIF- CSph, pSK-OCIF-CBsp and pSK-OCIF-CPst were designated as CBst DNA solution, CSph DNA solution, CBsp DNA solution and CPst DNA solution, respectively. One microliter of pCEP 4 DNA solution (described in EXAMPLE 22-ii)) and 6  $\mu$ l of either CBst DNA solution, CSph DNA solution, CBsp DNA solution or CPst DNA solution were independently mixed with 7  $\mu$ l of ligation buffer I of DNA ligation kit ver. 2 and ligation reactions were carried out. Competent E. coli DH5 $\alpha$  cells (100  $\mu$ l) were transformed with 7  $\mu$ l of each ligation mixture. Ampicillin-resistant transformants were screened for clones containing plasmids in which cDNA fragment is inserted between the recognition sites of Bam HI and Xho I of pCEP 4 by analyzing the DNA structure. The plasmids containing the cDNA encoding OCIF-CBst, OCIF-CSph, OCIF-CBsp and OCIF-CPst were designated as pCEP4-OCIF-CBst, pCEP4-OCIF-CSph, pCEP4-OCIF-CBsp and pCEP4-OCIF-CPst, respectively.

35 v) Preparation of vectors for expressing the OCIF mutants

40 E. coli clones harboring the expression vectors for OCIF mutants (total of 21 clones) were grown and the vectors were purified by QIAGEN column (QIAGEN). All the expression vectors were precipitated with ethanol and dissolved in appropriate volumes of sterile distilled water and used for further manipulations shown below.

45 vi) Transient expression of the cDNAs for OCIF mutants and biological activities of the mutants

50 OCIF mutants were produced using the expression vectors prepared in EXAMPLE 22-v). The method was essentially the same as described in EXAMPLE 13. Only the modified points are described below. A 24-well plate was used for the DNA transfection.  $2 \times 10^5$  cells of 293/EBNA suspended in IMDM containing 10% fetal bovine serum were seeded into each well of the plate. One microgram of purified vector DNA and 4  $\mu$ l of lipofectamine were used for each transfection. Mixture of an expression vector and lipofectamine in OPTI-MEM (GIBCO BRL) in a final volume of 0.5 ml was added to the cells in a well. After the cells were incubated at 37°C for 24 hr in a CO<sub>2</sub> incubator, the medium was replaced with 0.5 ml of Ex-cell 301 medium (JSR). The cells were incubated at 37 °C for 48 more hours in the CO<sub>2</sub> incubator. The conditioned medium was collected and used for assay for in vitro biological activity. The nucleotide

sequences of cDNAs for the OCIF mutants are shown in SEQUENCE NO:83-103. The deduced amino acid sequences for the OCIF mutants are shown in SEQUENCE NO: 62-82. The assay for in vitro biological activity was performed as described in EXAMPLE 13. Antigen concentration of each conditioned medium was determined by ELISA as described in EXAMPLE 24. Table 14 shows specific activity of the mutants relative to that of the unaltered OCIF.

5

Table 14

	mutants	activity
10	the unaltered OCIF	++
	OCIF-C19S	+
	OCIF-C20S	±
15	OCIF-C21S	±
	OCIF-C22S	+
	OCIF-C23S	++
	OCIF-DCR1	±
20	OCIF-DCR2	±
	OCIF-DCR3	±
	OCIF-DCR4	±
25	OCIF-DDD1	+
	OCIF-DDD2	±
	OCIF-CL	++
	OCIF-CC	++
30	OCIF-CDD2	++
	OCIF-CDD1	+
	OCIF-CCR4	±
35	OCIF-CCR3	±
	OCIF-CBst	++
	OCIF-CSph	++
	OCIF-CBsp	±
40	OCIF-CPst	±
++ indicates relative activity more than 50% of that of the unaltered OCIF + indicates relative activity between 10% and 50% ± indicates relative activity less than 10%, or production level too low to determine the accurate biological activity		

45

#### vii) western blot analysis

Ten microliters of the final conditioned medium was used for western blot analysis. Ten microliters of the sample were mixed with 10  $\mu$ l of SDS-PAGE sample buffer (0.5 M Tris-HCl, 20% glycerol, 4% SDS, 20 $\mu$ g/ml bromo phenol blue, pH 6.8) boiled for 3 min. and subjected to a 10 % SDS polyacryl amide gel electrophoresis under non-reducing conditions. After the electrophoresis, the separated proteins were blotted to PVDF membrane (ProBlott<sup>R</sup>, Perkin Elmer) using a semi-dry electroblotter (BIO-RAD). The membrane was incubated at 37°C with horseradish peroxidase labeled anti-OCIF antibodies for 2 hr. After the membrane was washed, protein bands which react with the labeled antibodies were detected using ECL system (Amersham). Two protein bands with approximate molecular masses of 60kD and 120kD were detected for the unaltered OCIF. On the other hand, almost exclusively 60kD protein band was detected for OCIF-C23S, OCIF-CL and OCIF CC. Protein bands with an approximate masses of 40kD-50kD and 30kD-40kD were the major ones for OCIF-CDD2 and OCIF-CDD1, respectively. These results indicate that Cys at 379 is responsible for the dimer formation, both the monomers and the dimers maintain the biological activity and a deletion of residues from Asp

at 177 to Leu at 380 does not abolish the biological activity of OCIF (positions of the amino acid resare shown in SEQUENCE NO: 4).

## EXAMPLE 23

5

Isolation of human genomic OCIF gene

10

i) Screening of a human genomic library

An amplified human placenta genomic library in Lambda FIX II vector purchased from STRATAGENE was screened for the gene encoding human OCIF using the human OCIF cDNA as a probe. Essentially, screening was done according to the instruction manual supplied with the genomic library. The basic protocols described in Molecular Cloning: A Laboratory Manual also were employed to manipulate phage, *E. coli*, and DNA.

The library was titered, and  $1 \times 10^6$  pfu of phage was mixed with XL1-Blue MRA host *E. coli* cells and plated on 20 plates (9 cm x 13 cm) with 9 ml per plate of top agarose. The plates were incubated overnight at 37°C. Filter plaque lifts were prepared using Hybond-N nylon membranes (Amersham). The membranes were processed by denaturation in a solution containing 1.5 M NaCl and 0.5 M NaOH for 1 minute at room temperature. The membranes were then neutralized by placing successively for one minute each in 1 M Tris-HCl (pH 7.5) and a solution containing 1.5 M NaCl and 0.5 M Tris-HCl (pH 7.5). The membranes were then transferred onto a filter paper wet with 2xSSC. Phage DNA was fixed

20 on the membranes with 1200  $\mu$ Joules of UV energy in STRATALINKER UV crosslinker 2400 (STRATAGENE) and the membranes were air dried. The membranes were immersed in Rapid Hybridization buffer (Amersham) and incubated for one hour at 65 °C before hybridization with  $^{32}$ P-labeled cDNA probe in the same buffer overnight at 65°C. Screening probe was prepared by labeling the OCIF cDNA with  $^{32}$ P using the Megaprime DNA labeling system (Amersham). Approximately,  $5 \times 10^5$  cpm probe was used for each ml of hybridization buffer. After the hybridization, the membranes

25 were rinsed in 2xSSC for five minutes at room temperature. The membranes were then washed four times, 20 minutes each time, in 0.5xSSC containing 0.1 % SDS at 65 °C. After the final wash, the membranes were dried and subjected to autoradiography at -80 °C with SUPER HR-H X-ray film (FUJI PFOTO FILM Co., Ltd.) and an intensifying screen. Upon examination of the autoradiograms, six positive signals were detected. Agar plugs were picked from the regions corresponded to these signals for phage purification. Each agar plug was soaked overnight in 0.5 ml of SM buffer con-

30 taining 1% chloroform to extract phage. Each extract containing phage was diluted 1000 fold with SM buffer and an aliquot of 1 ml or 20 ml was mixed with host *E. coli* described above. The mixture was plated on agar plates with top agarose as described above. The plates were incubated overnight at 37 °C, and filter lifts were prepared, prehybridized, hybridized, washed and autoradiographed as described above. This process of phage purification was applied to all six positive signals initially detected on the autoradiograms and was repeated until all phage plaques on agar plates hybrid-

35 ize with the cDNA probe. After purification, agar plugs of each phage isolate were soaked in SM buffer containing 1% chloroform and stored at 4 °C. Six individual phage isolates were designated  $\lambda$ OIF3,  $\lambda$ OIF8,  $\lambda$ OIF9,  $\lambda$ OIF11,  $\lambda$ OIF12 and  $\lambda$ OIF17, respectively.

40

ii) Analysis of the genomic clones by restriction enzyme digestion and Southern blot hybridization

45

DNA was prepared from each phage isolate by the plate lysate method as described in Molecular Cloning: A Laboratory Manual. DNA prepared from each phage was digested with restriction enzymes and the fragments derived from the digestion were separated on agarose gels. The fragments were then transferred to nylon membranes and subjected to Southern blot hybridization using OCIF cDNA as a probe. The results of the analysis revealed that the six phage isolates are individual clones. Among these fragments derived from the restriction enzyme digestion, those fragments which hybridized with the OCIF cDNA probe were subcloned into plasmid vectors and subjected to the nucleotide sequence analysis as described below.

50

iii) Subcloning restriction fragments derived from genomic clones into plasmid vectors and determination of the nucleotide sequence.

$\lambda$ OIF8 DNA was digested with restriction enzymes EcoRI and NotI, and the DNA fragments derived these from were separated on a 0.7% agarose gel. The 5.8 kilobase pairs (kb) EcoRI/NotI fragment was extracted from the gel using QIAEX II Gel Extraction Kit (QIAGEN) according to the procedure recommended by the manufacturer. The 5.8 kb EcoRI/NotI fragment was ligated with pBluescript II SK+ vector (STRATAGENE) which had been linearized with restriction enzymes EcoRI and NotI, using Ready-To-Go T4 DNA Ligase (Pharmacia) according to the procedure recommended by the manufacturer. Competent DH5  $\alpha$  *E. coli* cells (Amersham) were transformed with the recombinant plasmid and transformants were selected on L-plates containing 50  $\mu$ g/ml  $\beta$ -ampicillin. A clone harboring the recom-

EXAMPLE 26

Therapeutic effect on osteoporosis

5 (1) Method

Male Fischer rats, 6 weeks-old, were subjected to denervation of left forelimb. These rats were assigned to four groups(10 rats/group) and treated as follows ; group A, sham operated rats without administration ; group B, denervated rats with intravenous administration of vehicle ; group C, denervated rats administered OCIF intravenously at a dose of 5  $\mu$ g/kg twice a day ; group D, denervated rats administered OCIF intravenously at a dose of 50  $\mu$ g/kg twice a day. After denervation, OCIF was administered daily for 14 days. After 2 weeks treatment, the animals were sacrificed and their forelimbs were dissected. Thereafter bones were tested for mechanical strength.

15 (2) Results

Decrease of bone strength was observed in the animals of control groups as compared to those animals of the normal groups while bone strength was increase in the groups of animal received 50 mg of OCIF per kg body weight.

20 Industrial availability

The present invention provides both a novel protein which inhibits formation of osteoclasts and a efficient procedure to produce the protein. The protein of the present invention has an activity to inhibit formation of osteoclasts. The protein will be useful for the treatment of many diseases accompanying bone loss, such as osteoporosis, and as an antigen to be used for the immunological diagnosis of such diseases.

25 Referring to the deposited the microorgainsm

Name and Address of the Depositary Authority

30 Name: National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology Ministry of International Trade and Industry

Address: 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken 305, JAPAN

Deposited date: June 21, 1995

(It was transferred from Bikkoken No. P-14998, which was deposited on June 21, 1995.

35 Transferred date: October 25, 1995)

Accession Number: FERM BP-5267

40

45

50

55

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: SNOW BRANDS MILK PRODUCTS CO., LTD.
- (B) STREET:
- (C) CITY:
- (D) STATE:
- (E) COUNTRY:
- (F) POSTAL CODE (ZIP):
- (G) TELEPHONE:
- (H) TELEFAX:
- (I) TELEX:

(ii) TITLE OF INVENTION: Novel proteins and methods for producing the  
proteins

(iii) NUMBER OF SEQUENCES: 105

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER:
- (C) OPERATING SYSTEM:
- (D) SOFTWARE: Wordperfect windows

(v) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: JP
- (B) FILE REFERENCE:
- (C) FILING DATE:

40

45

50

55

(2) INFORMATION FOR SEQUENCE ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 6

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 1:

Xaa Tyr His Phe Pro Lys

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 14

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:2:

Xaa Gln His Ser Xaa Gln Glu Gln Thr Phe Gln Leu Xaa Lys

1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 12 .

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide (an internal amino acid sequence of the protein)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 3:

Xaa Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys

1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 380

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF protein without signal peptide)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:4:

Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His	Tyr	Asp	Glu	Glu	Thr	Ser
1								10					15	
His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro	Pro	Gly	Thr	Tyr	Leu	Lys
								20			25		30	
Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr	Val	Cys	Ala	Pro	Cys	Pro
								35			40		45	
Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His	Thr	Asp	Glu	Cys	Leu	
								50			55		60	
Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu	Gln	Tyr	Val	Lys	Gln	Glu
								65			70		75	
Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys	Glu	Cys	Lys	Glu	Gly	Arg
								80			85		90	
Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys	His	Arg	Ser	Cys	Pro	Pro
								95			100		105	
Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr	Pro	Glu	Arg	Asn	Thr	Val
								110			115		120	
Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe	Ser	Asn	Glu	Thr	Ser	Ser
								125			130		135	
Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn	Cys	Ser	Val	Phe	Gly	Leu
								140			145		150	
Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr	His	Asp	Asn	Ile	Cys	Ser
								155			160		165	
Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu
								170			175		180	
Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr
								185			190		195	
Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys
								200			205		210	
Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser
								215			220		225	
Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn
								230			235		240	

Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu		
245	250	255
Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr		
260	265	270
Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys		
275	280	285
Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro		
290	295	300
Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn		
305	310	315
Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His		
320	325	330
Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys		
335	340	345
Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr		
350	355	360
Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val		
365	370	375
Lys Ile Ser Cys Leu		
380		

(2) INFORMATION FOR SEQUENCE ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF protein with signal peptide)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 5:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser			
-20	-15	-10	
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His			
-5	-1 1	5	
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro			
10	15	20	
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr			
25	30	35	
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His			

	40	45	50
5	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
10	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
15	100	105	110
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
20	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
25	160	165	170
	Gly Ile Asp Val Thr Leu Cys Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
30	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
35	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
40	235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
	250	255	260
	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
45	265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
	280	285	290
50	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser		
	295	300	305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		

310	315	320
5	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr	
325	330	335
10	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe	
340	345	350
15	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly	
355	360	365
20	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
370	375	380

## (2) INFORMATION FOR SEQUENCE ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 6:

30 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCTCTCAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCTCTCTGG TACCTACCTA AAACAAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
 35 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAGA GOGAAATACA 420  
 40 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCGAG TGTCTTTGGT CTCTCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTCTACAA AGTTTACGCC TAACTGGCTT 660  
 45 AGTGTCTTGG TAGACAATTG GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 50 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCAGA TAAAAATGG CGACCAAGAC 1020

5 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCCAAACT 1080  
GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
TATCAGAAGT TATTTTTAGA AATGATAGGT AACCAAGTCC AATCAGTAAA AATAAGCTGC 1200  
TTATAA 1206

10 (2) INFORMATION FOR SEQUENCE ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 15
- (B) TYPE : amino acid
- (D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : peptide (a N-terminal amino acid sequence of the  
protein)

20 (xi) SEQUENCE DESCRIPTION :SEQ ID NO:7:

Glu Thr Phe Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser

1 5 10 15

25 (2) INFORMATION FOR SEQUENCE NO ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1185
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

30 (ii) MOLECULE TYPE : cDNA (OCIF2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO:8

35 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
40 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACCGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGTGC AATCGCACCC ACAACCGCGT GTGCGAATGC 300  
AAGGAAGGGC GCTACCTTGA GATAGAGTTC TGCTTGAAAC ATAGGAGCTG CCCTCCTGGA 360  
45 TTGGGAGTGG TGCAAGCTGG AACCCCAGAG CGAAATACAG TTTGCAAAG ATGTCAGAT 420  
GGGTTCTTCT CAAATGAGAC GTCATCTAAA GCACCCCTGTA GAAAACACAC AAATTGCACT 480  
GTCTTTGGTC TCCTGCTAAC TCAGAAAGGA AATGCAACAC ACGACAACAT ATGTTCCGGA 540  
50 AACAGTGAAT CAACTCAAAA ATGTGGAATA GATGTTACCC TGTGTGAGGA GGCATTCTTC 600  
AGGTTTGCTG TTCCCTACAAA GTTACGCTT AACTGGCTTA GTGTCTTGGT AGACAATTG 660  
CCTGGCACCA AAGTAAACGC AGAGAGTGTGAGAGGATAA AACGGCAACA CAGCTCACAA 720

5 GAACAGACTT TCCAGCTGCT GAAGTTATGG AAACATCAAA ACAAAGACCA AGATATAGTC 780  
 AAGAAAGATCA TCCAAGATAT TGACCTCTGT GAAAACAGCG TGCAGGGCA CATTGGACAT 840  
 GCTAACCTCA CCTTCGAGCA GCTTCGTTGAC TTGATGGAAA GCTTACCGGG AAAGAAAGTG 900  
 GGAGCAGAAG ACATTGAAAA ACAATAAAAG GCATGCAAAC CCAGTGACCA GATCCTGAAG 960  
 10 CTGTCAGTT TGTGGCGAAT AAAAAATGGC GACCAAGACA CCTTGAGGG CCTAATGCAC 1020  
 GCACAAAGC ACTCAAAGAC GTACCACTTT CCCAAACTG TCACTCAGAG TCTAAAGAAG 1080  
 ACCATCAGGT TCCTTCACAG CTTCACAAATG TACAAATTGT ATCAGAAAGTT ATTTTAGAA 1140  
 ATGATAGGTA ACCAGGTCCA ATCAGTAAAA ATAAGCTGCT TATAA 1185

## 15 (2) INFORMATION FOR SEQUENCE ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 394

(B) TYPE : amino acid

(D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF2)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

25 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5  
 30 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 35 25 30 35  
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 40 45 50  
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Cys  
 45 55 60 65  
 Asn Arg Thr His Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr  
 50 70 75 80  
 45 Leu Glu Ile Glu Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly  
 85 90 95  
 Phe Gly Val Val Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys  
 50 100 105 110  
 Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys  
 115 120 125

	Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu		
5	130	135	140
	Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly		
	145	150	155
	Asn Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys		
10	160	165	170
	Glu Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro		
	175	180	185
	Asn Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val		
15	190	195	200
	Asn Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln		
	205	210	215
20	Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys		
	220	225	230
	Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys		
	235	240	245
25	Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe		
	250	255	260
	Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val		
30	265	270	275
	Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser		
	280	285	290
	Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly		
35	295	300	305
	Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser		
	310	315	320
40	Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys		
	325	330	335
	Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln		
	340	345	350
45	Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys		
	355	360	365
	Ile Ser Cys Leu		
50	370	373	

(2) INFORMATION FOR SEQUENCE ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1089
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF3)

## (xi) SEQUENCE DESCRIPTION ID NO: 10:

10	ATGAAACAAGT	TGCTGTGCTG	CGCGCTCGTG	TTTCTGGACA	TCTCCATTAA	GTGGACCACC	60
15	CAGGAAACGT	TTCCCTCCAAA	GTACCTTCAT	TATGACGAAG	AAACCTCTCA	TCAGCTGTTG	120
20	TGTGACAAAT	GTCCTCCTGG	TACCTACCTA	AAACAACACT	GTACAGCAAA	GTGGAAGACC	180
25	GTGTGCGCCC	CTTGCCTCTGA	CCACTACTAC	ACAGACAGCT	GGCACACCCAG	TGACGAGTGT	240
30	CTATACTGCA	GCCCCGTGTG	CAAGGAGCTG	CAGTACGTCA	AGCAGGAGTG	CAATCGCACC	300
35	CACAACCGCG	TGTGCGAATG	CAAGGAAGGG	CGCTACCTTG	AGATAGAGTT	CTGCTTGAAA	360
40	CATAGGAGCT	GCCCTCCTGG	ATTTGGAGTG	GTGCAAGCTG	GAACCCCGAGA	GCGAAATACA	420
45	GTTTGCAAAA	GATGTCCAGA	TGGGTTCTTC	TCAAATGAGA	CGTCATCTAA	AGCACCCCTGT	480
50	AGAAAACACA	CAAATTGAG	TGTCTTGGT	CTCCTGCTAA	CTCAGAAAGG	AAATGCAACA	540
55	CACGACAACA	TATGTTCCGG	AAACAGTGAA	TCAACTCAA	AATGTGGAAT	AGATGTTACC	600
60	CTGTGTGAGG	AGGCATTCTT	CAGGTTTGCT	GTTCTACAA	AGTTTACGCC	TAACTGGCTT	660
65	AGTGTCTTGG	TAGACAATT	GCCTGGCACC	AAAGTAAACG	CAGAGAGTGT	AGAGAGGATA	720
70	AAACGGCAAC	ACAGCTCACA	AGAACAGACT	TTCCAGCTGC	TGAAGTTATG	GAAACATCAA	780
75	AACAAAGACC	AAGATATAGT	CAAGAAGATC	ATCCAAGATA	TTGACCTCTG	TGAAAACAGC	840
80	GTGCAGCGGC	ACATTGGACA	TGCTAACCTC	AGTTTGTGGC	GAATAAAAAA	TGGCGACCAA	900
85	GACACCTTGA	AGGGCTTAAT	GCACGCACTA	AAGCACTCAA	AGACGTACCA	CTTICCCAAA	960
90	ACTGTCACTC	AGAGTCTAAA	GAAGACCATC	AGGTTCTTC	ACAGCTTCAC	AATGTACAAA	1020
95	TTGTATCAGA	AGTTATTTT	AGAAATGATA	GGTAACCAGG	TCCAATCACT	AAAAATAAGC	1080
	TGCTTATAA						1089

## (2) INFORMATION FOR SEQUENCE ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 362
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF3)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Asn Lys Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

	-20	-15	-10
5	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1	5
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
10	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His		
	40	45	50
15	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
20	70	75	80
	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
25	100	105	110
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
30	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
35	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
40	175	180	185
	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
45	205	210	215
	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
50	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
	235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		

250 255 260  
Gly His Ala Asn Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln  
5 265 270 275  
Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr  
280 285 290  
10 Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile  
295 300 305  
Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu  
310 315 320  
15 Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser  
325 330 335  
Cys Leu  
20 340 341

(2) INFORMATION FOR SEQUENCE ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH : 465  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
30 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 12:

ATGAACAAAGT TGCTGTGCTG CTCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
35 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
40 GTGTGCGCOC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
45 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GTACGTGTCA ATGTGGCAGCA 420  
AAATTAATTA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAG 465

(2) INFORMATION FOR SEQUENCE ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH :154  
(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF4)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Asn Lys Leu Leu Cys Cys Ser Leu Val Phe Leu Asp Ile Ser		
10 -20	-15	-0
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
15 -5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
20 10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25 25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
30 40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
35 55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
40 70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
45 85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
50 100	105	110
Cys Gln Cys Ala Ala Lys Leu Ile Arg Ile Met Gln Ser Gln Ile		
55 115	120	125
Val Val Thr Val		
60 130	133	

(2) INFORMATION FOR SEQUENCE ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 438

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF5)

(xi) SEQUENCE DESCRIPTION ID NO: 14:

ATGAACAAAGT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60

CAGGAAACGT TTCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCAACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GGCCTCTGG ATTGGAGTG GTGCAAGCTG GATGCAGGAG AAGACCCAAG 420  
CCACAGATAT GTATCTGA 438

(2) INFORMATION FOR SEQUENCE ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 140
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCTE5)

(xi) SEQUENCE DESCRIPTION: TD NO: 15:

(2) INFORMATION FOR SEQUENCE ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer T3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AATTAACCCCT CACTAAAGGG

20

15

(2) INFORMATION FOR SEQUENCE ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH : 22
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer T7)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GTAATACGAC TCACTATAGG GC

22

30

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : synthetic DNA (primer IF1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 18:

ACATCAAAAC AAAGACCAAG

20

40

(2) INFORMATION FOR SEQUENCE ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

55

5 (ii) MOLECULE TYPE : synthetic DNA (primer IF2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 19:

TCTTGGTCTT TGTTTGATG

20

10 (2) INFORMATION FOR SEQUENCE ID NO: 20:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

20 (ii) MOLECULE TYPE : synthetic DNA (primer IF3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 20:

25 TTATTCGCCA CAAACTGAGC

20

30 (2) INFORMATION FOR SEQUENCE ID NO: 21:

35 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer IF4)

45 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 21:

50 TTGTGAAGCT GTGAAGGAAC

20

(2) INFORMATION FOR SEQUENCE ID NO: 22:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

45 (ii) MOLECULE TYPE : synthetic DNA (primer IF5)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 22:

50 GCTCAGTTG TGGCGAATAA

20

55 (2) INFORMATION FOR SEQUENCE ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF6)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 23:

GTGGGAGGCAG AAGACATTGA

20

(2) INFORMATION FOR SEQUENCE ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF7)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 24:

AATGAACAAAC TTGCTGTGCT

20

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF8)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 25:

TGACAAATGT CCTCCTGGTA

20

(2) INFORMATION FOR SEQUENCE ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer IF9)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 26:

AGGTAGGTAC CAGGAGGACA

20

5

(2) INFORMATION FOR SEQUENCE ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

15

(ii) MOLECULE TYPE : synthetic DNA (primer IF10)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 27:

GAGCTGCCCT CCTGGATTG

20

20

(2) INFORMATION FOR SEQUENCE ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

30

(ii) MOLECULE TYPE : synthetic DNA (primer IF11)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 28:

CAAACGTGAT TTCGCTCTGG

20

35

(2) INFORMATION FOR SEQUENCE ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

45

(ii) MOLECULE TYPE : synthetic DNA (primer IF12)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 29:

GTGTGAGGAG GCATTCTTCA

20

50

(2) INFORMATION FOR SEQUENCE ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

55

- (A) LENGTH : 32

5 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer C19SF)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 30:

10 GAATCAACTC AAAAAAGTGG AATAGATGTT AC

32

15 (2) INFORMATION FOR SEQUENCE ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH : 32  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

20 (ii) MOLECULE TYPE : synthetic DNA (primer C19SR)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 31:

25 GTAACATCTA TTCCACTTTT TTGAGTTGAT TC

32

30 (2) INFORMATION FOR SEQUENCE ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH : 30  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

35 (ii) MOLECULE TYPE : synthetic DNA (primer C20SF)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 32:

40 ATAGATGTTA CCCTGAGTGA GGAGGCATTC

30

45 (2) INFORMATION FOR SEQUENCE ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH : 30  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

50 (ii) MOLECULE TYPE : synthetic DNA (primer C20SR)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 33:

GAATGCCTCC TCACTCAGGG TAACATCTAT

30

5 (2) INFORMATION FOR SEQUENCE ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer C21SF)

15 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 34:

CAAGATATTG ACCTCAGTGA AAACAGCGTG C

31

20 (2) INFORMATION FOR SEQUENCE ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer C21SR)

30 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 35:

GCACGCTGTT TTCACTGAGG GCAATACTT G

31

35 (2) INFORMATION FOR SEQUENCE ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer C22SF)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 36:

45 AAAACAATAA AGGCAAGCAA ACCCAGTGAC C

31

50 (2) INFORMATION FOR SEQUENCE ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 31
- (B) TYPE : nucleic acid

5 (C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C22SR)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 37:  
GGTCACTGGG TTGCTTGCC TTTATTGTTT T 31

10 (2) INFORMATION FOR SEQUENCE ID NO: 38:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 31  
15 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C23SF)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 38:  
TCAGTAAAAA TAAGCAGCTT ATAACGGCC A 31

20 (2) INFORMATION FOR SEQUENCE ID NO: 39:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 31  
25 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer C23SR)  
30 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 39:  
TGGCCAGTTA TAAGCTGCTT ATTTTACTG A 31

35 (2) INFORMATION FOR SEQUENCE ID NO: 40:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 22  
40 (B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : synthetic DNA (primer IF 14)  
45 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 40:  
TTGGGGTTA TTGGAGGAGA TG 22

50

5 (2) INFORMATION FOR SEQUENCE ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer DCR1F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 41:

15 ACCACCCAGG AACCTTGCCC TGACCACTAC TACACA

36

20 (2) INFORMATION FOR SEQUENCE ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer DCR1R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 42:

30 GTCAGGGCAA GGTTCCCTGGG TGGTCCACTT AATGGA

36

35 (2) INFORMATION FOR SEQUENCE ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer DCR2F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 43:

45 ACCGTGTGCG CGGAATGCAA GGAAGGGCGC TACCTT

36

50 (2) INFORMATION FOR SEQUENCE ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DCR2R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 44:

5 TTCCTTGCAT TCGGCGCACA CGGTCTTCCA CTTTGC

36

(2) INFORMATION FOR SEQUENCE ID NO: 45:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : synthetic DNA (primer DCR3F)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 45:

20 AACCGCGTGT GCAGATGTCC AGATGGGTTTC TTCTCA

36

(2) INFORMATION FOR SEQUENCE ID NO: 46:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

30 (ii) MOLECULE TYPE : synthetic DNA (primer DCR3R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 46:

35 ATCTGGACAT CTGCACACGC GGTTGTGGGT GCGATT

36

(2) INFORMATION FOR SEQUENCE ID NO: 47:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

45 (ii) MOLECULE TYPE : synthetic DNA (primer DCR4F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 47:

50 ACAGTTTGCA AATCOGGAAA CAGTGAATCA ACTCAA

36

(2) INFORMATION FOR SEQUENCE ID NO: 48:

55 (i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer DCR4R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 48:

ACTGTTTCCG GATTTGAAA CTGTATTCG CTCTGG

36

15 (2) INFORMATION FOR SEQUENCE ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer DDD1F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 49:

AATGTGGAAT AGATATTGAC CTCTGTGAAA ACAGCG

36

30 (2) INFORMATION FOR SEQUENCE ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer DDD1R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 50:

AGAGGTCAAT ATCTATTCCA CATTGGAG TTGATT

36

45 (2) INFORMATION FOR SEQUENCE ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH : 36  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer DDD2F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 51:

AGATCATCCA AGACGGACTA AAGCACTCAA AGACGT

36

5 (2) INFORMATION FOR SEQUENCE ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 36
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

10 (ii) MOLECULE TYPE : synthetic DNA (primer DDD2R)

15 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 52:

GCTTTAGTGC GTCTTGGATG ATCTTCTTGA CTATAT

36

20 (2) INFORMATION FOR SEQUENCE ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : synthetic DNA (primer XhoI F)

30 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 53:

GGCTCGAGCG CCCAGCCGCC GCCTCCAAG

29

35 (2) INFORMATION FOR SEQUENCE ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 20
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : synthetic DNA (primer IF 16)

45 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 54:

TTTGAGTGCT TTAGTGGGTG

20

50 (2) INFORMATION FOR SEQUENCE ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 30
- (B) TYPE : nucleic acid

55

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

6 (ii) MOLECULE TYPE : synthetic DNA (primer CL F)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 55:

TCAGTAAAAA TAAGCTAACT GGAAATGGCC

30

10

(2) INFORMATION FOR SEQUENCE ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 30

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

20

(ii) MOLECULE TYPE : synthetic DNA (primer CL R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 56:

GGCCATTTCAGTTAGCTTA TTTTACTGA

30

25

(2) INFORMATION FOR SEQUENCE ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 29

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

30

(ii) MOLECULE TYPE : synthetic DNA (primer CC R)

35

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 57:

CCGGATCCTC AGTGCTTAG TGCCTGCAT

29

40

(2) INFORMATION FOR SEQUENCE ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 29

(B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

45

(ii) MOLECULE TYPE : synthetic DNA (primer CCD2 R)

50

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 58:

CCGGATCCTC ATTGGATGAT CTTCTTGAC

29

55

(2) INFORMATION FOR SEQUENCE ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : synthetic DNA (primer CCD1 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 59:

CCGGATCCTC ATATTCCACA TTTTGAGT

29

15

(2) INFORMATION FOR SEQUENCE ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

20

(ii) MOLECULE TYPE : synthetic DNA (primer CCR4 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 60:

CCGGATCCTC ATTTGAAAC TGTATTCG

29

30

(2) INFORMATION FOR SEQUENCE ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 29
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

35

(ii) MOLECULE TYPE : synthetic DNA (primer CCR3 R)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 61:

CCGGATCCTC ATTCGCACAC GCGGTTGTG

29

40

(2) INFORMATION FOR SEQUENCE ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

55

(ii) MOLECULE TYPE : Protein (OCIF-C19S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 62:

5	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
	-20	-15	-10
10	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1	5
15	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
20	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
25	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
	40	45	50
30	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
35	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
40	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
45	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
50	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
55	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
60	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
65	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Ser		
	160	165	170
70	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
75	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
80	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
85	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230

	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile
5	235 240 245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile
	250 255 260
	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu
10	265 270 275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr
	280 285 290
15	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser
	295 300 305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu
	310 315 320
	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr
20	325 330 335
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe
	340 345 350
25	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly
	355 360 365
	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
30	370 375 380

(2) INFORMATION FOR SEQUENCE ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCTE-C20S)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 63:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
-5 -1 1 5

Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
10 15 20

Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr

25	30	35
5	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	
40	45	50
5	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	
55	60	65
10	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	
70	75	80
5	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	
85	90	95
15	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr	
100	105	110
5	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe	
115	120	125
20	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn	
130	135	140
5	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr	
145	150	155
25	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys	
160	165	170
5	Gly Ile Asp Val Thr Leu Ser Glu Glu Ala Phe Phe Arg Phe Ala	
175	180	185
30	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp	
190	195	200
5	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile	
205	210	215
35	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys	
220	225	230
40	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile	
235	240	245
45	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile	
250	255	260
50	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu	
265	270	275
55	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
280	285	290
55	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser	

295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
10 Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365
15 Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
370	375	380

## (2) INFORMATION FOR SEQUENCE ID NO: 64:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C21S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 64:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser			
-20	-15	-10	
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His			
-5	-1 1	5	
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro			
10	15	20	
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr			
25	30	35	
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His			
40	45	50	
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu			
55	60	65	
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys			
70	75	80	
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys			
85	90	95	

His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
 100 105 110  
 5 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
 115 120 125  
 Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
 130 135 140  
 10 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
 145 150 155  
 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
 15 160 165 170  
 16 Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala  
 175 180 185  
 20 Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp  
 190 195 200  
 Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile  
 205 210 215  
 25 Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys  
 220 225 230  
 Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile  
 235 240 245  
 30 Ile Gln Asp Ile Asp Leu Ser Glu Asn Ser Val Gln Arg His Ile  
 250 255 260  
 Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu  
 265 270 275  
 35 Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr  
 280 285 290  
 Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser  
 40 295 300 305  
 Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu  
 310 315 320  
 45 Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr  
 325 330 335  
 Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe  
 340 345 350  
 50 Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly  
 355 360 365

Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
370 375 380

6

(2) INFORMATION FOR SEQUENCE ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

10

(A) LENGTH : 401

(B) TYPE : amino acid

(C) **STRANDEDNESS** : single

(D) TOPOLOGY : Linear

15

(ii) MOLECULE TYPE : Protein (OCTE-C22S)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 65:

20

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20														
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5														
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10														
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25														
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40														
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
55														
Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
70														
Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
85														
His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
100														
Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
115														
Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
130														
Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
145														
His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys

55

160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
175	180	185
Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
190	195	200
Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Ser Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365
Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
370	375	380

## (2) INFORMATION FOR SEQUENCE ID NO: 66:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 401
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-C23S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 66:

5	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
	-20	-15	-10
10	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1 1	5
15	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
20	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
25	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
	40	45	50
30	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
35	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
	70	75	80
40	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
45	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
50	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
55	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
60	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
65	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
70	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
75	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
80	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
85	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230

	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
5	235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
	250	255	260
10	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
	265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
	280	285	290
15	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
	295	300	305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
	310	315	320
20	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
	325	330	335
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
	340	345	350
25	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
	355	360	365
	Asn Gln Val Gln Ser Val Lys Ile Ser Ser Leu		
30	370	375	380

(2) INFORMATION FOR SEQUENCE ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 360
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-DCR1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 67:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20													-10	
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr
-5													5	
Asp	Ser	Trp	His	Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val
10													20	
Cys	Lys	Glu	Leu	Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His

	25	30	35
5	Asn Arg Val Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu		
	40	45	50
	Phe Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val		
	55	60	65
10	Gln Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro		
	70	75	80
	Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg		
	85	90	95
15	Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys		
	100	105	110
	Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser		
20	115	120	125
	Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe		
	130	135	140
	Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser		
25	145	150	155
	Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser		
	160	165	170
30	Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe		
	175	180	185
	Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile		
	190	195	200
35	Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val		
	205	210	215
	Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg		
40	220	225	230
	Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp		
	235	240	245
	Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu		
45	250	255	260
	Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr		
	265	270	275
	Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His		
50	280	285	290
	Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe		

295	300	305
Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu		
310	315	320
Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
325	330	335

10

(2) INFORMATION FOR SEQUENCE ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 359
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

20 (ii) MOLECULE TYPE : Protein (OCIF-DCR2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 68:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe		
40	45	50
Cys Leu Lys His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln		
55	60	65
Ala Gly Thr Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp		
70	75	80
Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys		
85	90	95
His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly		
100	105	110
Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr		
115	120	125
Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe		
130	135	140

Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val  
 145 150 155  
 5 Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val  
 160 165 170  
 Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln  
 175 180 185  
 10 Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val  
 190 195 200  
 Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln  
 15 205 210 215  
 Arg His Ile Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser  
 220 225 230  
 20 Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile  
 235 240 245  
 Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys  
 250 255 260  
 25 Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu  
 265 270 275  
 Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe  
 30 280 285 290  
 Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu  
 295 300 305  
 His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu  
 35 310 315 320  
 Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu  
 325 330 335

40 (2) INFORMATION FOR SEQUENCE ID NO: 69:

45 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 363

50 (B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-DCR3)

55 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 69:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

	-20	-15	-10
	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
5	-5	-1	5
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
	10	15	20
10	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His		
	40	45	50
15	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
20	70	75	80
	Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser Lys Ala		
	85	90	95
25	Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu Leu		
	100	105	110
	Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn		
	115	120	125
30	Ser Glu Ser Thr Gln Lys Cys Gly Ile Asp Val Thr Leu Cys Glu		
	130	135	140
	Glu Ala Phe Phe Arg Phe Ala Val Pro Thr Lys Phe Thr Pro Asn		
	145	150	155
35	Trp Leu Ser Val Leu Val Asp Asn Leu Pro Gly Thr Lys Val Asn		
	160	165	170
	Ala Glu Ser Val Glu Arg Ile Lys Arg Gln His Ser Ser Gln Glu		
40	175	180	185
	Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His Gln Asn Lys Asp		
	190	195	200
	Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp Leu Cys Glu		
45	205	210	215
	Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu		
	220	225	230
50	Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly		
	235	240	245
	Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp		

250	255	260
Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp		
265	270	275
Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys		
280	285	290
Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr		
295	300	305
Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys		
310	315	320
Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile		
325	330	335
Ser Cys Leu		
340		

## (2) INFORMATION FOR SEQUENCE ID NO: 70:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 359
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF-DCR4)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 70:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1	1
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His		
40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
70	75	80

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	Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
5	85								90					95	
	His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
	100							105					110		
10	Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Ser	Gly	Asn	Ser	Glu	Ser	Thr
	115							120					125		
	Gln	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe
	130							135					140		
15	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val
	145							150					155		
	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val
	160							165					170		
20	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln
	175							180					185		
	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp	Gln	Asp	Ile	Val
	190							195					200		
25	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	Cys	Glu	Asn	Ser	Val	Gln
	205							210					215		
	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr	Phe	Glu	Gln	Leu	Arg	Ser
	220							225					230		
30	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys	Lys	Val	Gly	Ala	Glu	Asp	Ile
	235							240					245		
	Glu	Lys	Thr	Ile	Lys	Ala	Cys	Lys	Pro	Ser	Asp	Gln	Ile	Leu	Lys
	250							255					260		
35	Leu	Leu	Ser	Leu	Trp	Arg	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu
	265							270					275		
	Lys	Gly	Leu	Met	His	Ala	Leu	Lys	His	Ser	Lys	Thr	Tyr	His	Phe
	280							285					290		
40	Pro	Lys	Thr	Val	Thr	Gln	Ser	Leu	Lys	Lys	Thr	Ile	Arg	Phe	Leu
	295							300					305		
45	His	Ser	Phe	Thr	Met	Tyr	Lys	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu
	310							315					320		
	Met	Ile	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	Cys	Leu	
	325							330					335		

(2) INFORMATION FOR SEQUENCE ID NO: 71:

## (i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 326  
 (B) TYPE : amino acid  
 (C) STRANDEDNESS : single  
 (D) TOPOLOGY : linear

## 10 (ii) MOLECULE TYPE : protein (OCIF-DDD1)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 71:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10

15 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5

20 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20

25 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 30 35

30 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His  
 40 45 50

35 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
 55 60 65

40 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
 70 75 80

45 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
 85 90 95

50 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
 100 105 110

55 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
 115 120 125

60 Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
 130 135 140

65 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
 145 150 155

70 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
 160 165 170

75 Gly Ile Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile  
 175 180 185

80 Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu

190	195	200
5	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
205	210	215
	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser	
220	225	230
10	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu	
235	240	245
	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr	
250	255	260
15	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe	
265	270	275
	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly	
280	285	290
20	Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu	
295	300	305

## 25 (2) INFORMATION FOR SEQUENCE ID NO: 72:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 327
- (B) TYPE: amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : protein (OCIF-DDD2)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 72:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
30		
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
35		
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10	15	20
40		
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25	30	35
45		
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His		
40	45	50
50		
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
55	60	65

	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
5	70	75	80
	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
10	100	105	110
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
15	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
20	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
25	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
30	205	210	215
	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
35	235	240	245
	Ile Gln Asp Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys		
	250	255	260
40	Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser		
	265	270	275
	Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile		
	280	285	290
45	Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu		
	295	300	305

## (2) INFORMATION FOR SEQUENCE ID NO: 73:

50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH : 399

(B) TYPE : amino acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : protein (OCIF-CL)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 73:

10	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser			
	-20	-15	-10	
15	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His			
	-5	-1	1	5
20	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro			
	10	15	20	
25	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr			
	25	30	35	
30	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His			
	40	45	50	
35	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu			
	55	60	65	
40	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys			
	70	75	80	
45	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys			
	85	90	95	
50	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr			
	100	105	110	
55	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe			
	115	120	125	
60	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn			
	130	135	140	
65	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr			
	145	150	155	
70	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys			
	160	165	170	
75	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala			
	175	180	185	
80	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp			
	190	195	200	
85	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile			

205	210	215
Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
220	225	230
Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
235	240	245
Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
250	255	260
Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
265	270	275
Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
280	285	290
Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
295	300	305
Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
310	315	320
Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
325	330	335
Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
340	345	350
Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
355	360	365
Asn Gln Val Gln Ser Val Lys Ile Ser		
370	375	

35

## (2) INFORMATION FOR SEQUENCE ID NO: 74:

## (i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH : 351
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

45

## (ii) MOLECULE TYPE : protein (OCIF-CC)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 74:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
---	--	--

-20	-15	-10
-----	-----	-----

50

Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
---	--	--

-5	-1	1	5
----	----	---	---

	Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
5	10														
	Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
	25														
	Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
10	40														
	Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Cys	Ser	Pro	Val	Cys	Lys	Glu	Leu
	55														
	Gln	Tyr	Val	Lys	Gln	Glu	Cys	Asn	Arg	Thr	His	Asn	Arg	Val	Cys
15	70														
	Glu	Cys	Lys	Glu	Gly	Arg	Tyr	Leu	Glu	Ile	Glu	Phe	Cys	Leu	Lys
	85														
	His	Arg	Ser	Cys	Pro	Pro	Gly	Phe	Gly	Val	Val	Gln	Ala	Gly	Thr
20	100														
	Pro	Glu	Arg	Asn	Thr	Val	Cys	Lys	Arg	Cys	Pro	Asp	Gly	Phe	Phe
	115														
25	Ser	Asn	Glu	Thr	Ser	Ser	Lys	Ala	Pro	Cys	Arg	Lys	His	Thr	Asn
	130														
	Cys	Ser	Val	Phe	Gly	Leu	Leu	Leu	Thr	Gln	Lys	Gly	Asn	Ala	Thr
30	145														
	His	Asp	Asn	Ile	Cys	Ser	Gly	Asn	Ser	Glu	Ser	Thr	Gln	Lys	Cys
	160														
	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe	Arg	Phe	Ala
35	175														
	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp
	190														
	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile
40	205														
	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys
	220														
	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Lys	Ile
45	235														
	Ile	Gln	Asp	Ile	Asp	Leu	Cys	Glu	Asn	Ser	Val	Gln	Arg	His	Ile
	250														
50	Gly	His	Ala	Asn	Leu	Thr	Phe	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu
	265														

Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr  
 280 285 290  
 5 Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Ser  
 295 300 305  
 Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu  
 10 310 315 320  
 Met His Ala Leu Lys His  
 325 330

## (2) INFORMATION FOR SEQUENCE ID NO: 75:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 272
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : Protein (OCIF-CDD2)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 75:

25 Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 30 -5 -1 1 5  
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 35 25 30 35  
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 40 45 50  
 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
 45 55 60 65  
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
 70 75 80  
 50 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
 85 90 95  
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
 100 105 110  
 Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe  
 115 120 125

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
 130 135 140  
 5 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
 145 150 155  
 His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys  
 10 160 165 170  
 Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala  
 175 180 185  
 15 Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp  
 190 195 200  
 Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile  
 205 210 215  
 20 Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys  
 220 225 230  
 Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile  
 235 240 245  
 25 Ile Gln  
 250

30 (2) INFORMATION FOR SEQUENCE ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 197
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CDD1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 76:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 40 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5  
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 45 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 30 35  
 50 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His

40	45	50
5	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	
55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	
70	75	80
10	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Ieu Lys	
85	90	95
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr	
100	105	110
15	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe	
115	120	125
	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn	
130	135	140
20	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr	
145	150	155
	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys	
25	160	165
	Gly Ile	170
	175	

30 (2) INFORMATION FOR SEQUENCE ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 143
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 77:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser		
-20	-15	-10
45 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
50 10 15 20		
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
25 30 35		

Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 40 45 50  
 5 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
 55 60 65  
 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
 10 70 75 80  
 15 Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys  
 85 90 95  
 His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr  
 100 105 110  
 Pro Glu Arg Asn Thr Val Cys Lys  
 115 120

## 20 (2) INFORMATION FOR SEQUENCE ID NO: 78:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 106
- (B) TYPE : amino acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## 30 (ii) MOLECULE TYPE : Protein (OCIF-CCR3)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 78:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser  
 -20 -15 -10  
 35 Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His  
 -5 -1 1 5  
 Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro  
 10 15 20  
 40 Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr  
 25 30 35  
 Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His  
 40 45 50  
 45 Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu  
 55 60 65  
 50 Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys  
 70 75 80  
 Glu

(2) INFORMATION FOR SEQUENCE ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 393

(B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CBst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 79:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser	-20	-15	-10
Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His	-5	-1 1	5
Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro	10	15	20
Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr	25	30	35
Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His	40	45	50
Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu	55	60	65
Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys	70	75	80
Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys	85	90	95
His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr	100	105	110
Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe	115	120	125
Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn	130	135	140
Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr	145	150	155
His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys	160	165	170
Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala			

	175	180	185
5	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
10	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
	235	240	245
15	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		
	250	255	260
	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu		
20	265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr		
	280	285	290
25	Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser		
	295	300	305
	Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu		
	310	315	320
30	Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr		
	325	330	335
	Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe		
	340	345	350
35	Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly		
	355	360	365
	Asn Leu Val		
40	370		

## (2) INFORMATION FOR SEQUENCE ID NO: 80:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 321

(B) TYPE : amino acid

(D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : Protein (OCIF-CSph)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 80:

Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser

	-20	-15	-10
5	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His		
	-5	-1	1
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro		
10		15	20
15	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr		
	25	30	35
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Asp Ser Trp His		
	40	45	50
20	Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys Glu Leu		
	55	60	65
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys		
25	70	75	80
	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys		
	85	90	95
30	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr		
	100	105	110
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe		
	115	120	125
35	Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn		
	130	135	140
	Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr		
	145	150	155
40	His Asp Asn Ile Cys Ser Gly Asn Ser Glu Ser Thr Gln Lys Cys		
	160	165	170
	Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe Ala		
	175	180	185
45	Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp		
	190	195	200
	Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile		
	205	210	215
50	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys		
	220	225	230
	Leu Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile		
	235	240	245
	Ile Gln Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile		

250	255	260
5	Gly His Ala Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu	
265	270	275
	Ser Leu Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr	
280	285	290
10	Ile Lys Ala Ser Leu Asp	
295	300	

## (2) INFORMATION FOR SEQUENCE ID NO: 81:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 202
- (B) TYPE : amino acid
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIF-CBsp)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 81:

25	Met Asn Asn Leu Leu Cys Cys Ala Leu Val Phe Leu Asp Ile Ser			
	-20	-15	-10	
	Ile Lys Trp Thr Thr Gln Glu Thr Phe Pro Pro Lys Tyr Leu His			
20	-5	-1	1	5
30	10	15	20	
	Tyr Asp Glu Glu Thr Ser His Gln Leu Leu Cys Asp Lys Cys Pro			
35	25	30	35	
	Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala Lys Trp Lys Thr			
40	40	45	50	
	Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp Ser Trp His			
45	55	60	65	
50	70	75	80	
	Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val Cys			
55	85	90	95	
	Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys			
60	100	105	110	
	His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala Gly Thr			
65	115	120	125	
	Pro Glu Arg Asn Thr Val Cys Lys Arg Cys Pro Asp Gly Phe Phe			
70	130	135	140	

Ser Asn Glu Thr Ser Ser Lys Ala Pro Cys Arg Lys His Thr Asn  
 145 150 155  
 Cys Ser Val Phe Gly Leu Leu Leu Thr Gln Lys Gly Asn Ala Thr  
 160 165 170  
 His Asp Asn Ile Cys Ser Gly  
 175 180

(2) INFORMATION FOR SEQUENCE ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 84
- (B) TYPE : amino acid
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : Protein (OCIE-CPst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 82:

Met	Asn	Asn	Leu	Leu	Cys	Cys	Ala	Leu	Val	Phe	Leu	Asp	Ile	Ser
-20													-10	
Ile	Lys	Trp	Thr	Thr	Gln	Glu	Thr	Phe	Pro	Pro	Lys	Tyr	Leu	His
-5													5	
Tyr	Asp	Glu	Glu	Thr	Ser	His	Gln	Leu	Leu	Cys	Asp	Lys	Cys	Pro
10													20	
Pro	Gly	Thr	Tyr	Leu	Lys	Gln	His	Cys	Thr	Ala	Lys	Trp	Lys	Thr
25													35	
Val	Cys	Ala	Pro	Cys	Pro	Asp	His	Tyr	Tyr	Thr	Asp	Ser	Trp	His
40													50	
Thr	Ser	Asp	Glu	Cys	Leu	Tyr	Leu	Val						
55														
					60						63			

(2) INFORMATION FOR SEQUENCE ID NO: 82:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCT1-C19S)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 83:

5 ATGAAACAAT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 10 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCTGG ATTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
 GTTTGCAGAA GATGTCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 15 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AAAGTGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCCTACAA AGTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 20 AACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAATGCAA 960  
 25 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCAACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
 30 TATCAGAACT TATTTTTAGA AATGATAGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 TTATAA 1206

## (2) INFORMATION FOR SEQUENCE ID NO: 84:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF-C20S)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 84:

45 ATGAAACAAT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
 CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300

CACAACCCGG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
AGAAAACACA CAAATTGCAG TGTCTTGCT CTCCGTCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600  
CTGAGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTACAA AGTTTACGCC TAACTGGCTT 660  
AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
AACAAAGACC AAGATATACT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
AGCTTACCCG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960  
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTCACA GCTTCACAAT GTACAAATTG 1140  
TATCAGAAGT TATTTTACA AATGATAGGT AACCAAGGTCC AATCAGTAAA AATAAGCTGC 1200  
TTATAA 1206

(2) INFORMATION FOR SEQUENCE ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C21S)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 85:

ATGAAACAAT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTAOctA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCAACACCAG TGACGAGTGT 240  
CTATACTGCA GCCCGGTGTG CAAGGAGCTG CAGTACGTCA ACCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCOCAGA GCGAAATACA 420  
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGTG 480  
AGAAAAACACA CAAATTGCAAG TGTCTTGGT CTCCGTCAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA ATGTGGAAT AGATGTTACC 600

5 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGACAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCAG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 10 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCTTCACA GCTTCACAAT GTACAAATTG 1140  
 15 TATCAGAACT TATTTTTAGA AATGATAGGT AACCAAGGTCC AATCACTAAA AATAAGCTGC 1200  
 TTATAA 1206

20 (2) INFORMATION FOR SEQUENCE ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-C22S)

(xi) SEQUENCE DESCRIPTION : SEQ ID NO: 86:

35 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCTTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGGCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 40 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAAG TGTCTTGGT CTCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 45 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCTACAA AGTTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 50 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900

AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAAGCAAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 5 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCTTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCTGC 1200  
 10 TTATAA 1206

## (2) INFORMATION FOR SEQUENCE ID NO: 87:

## (i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH : 1206
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- 20 (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF-C23S)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 87:

25 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTG 120  
 TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 30 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAAGA GCGAAATACA 420  
 35 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAAG TGTCTTTGGT CTCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 40 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTACAA AGTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 45 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCAAGCAAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 50 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCTTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTAGA AATGATAGGT AACCAGGTCC AATCAGTAAA AATAAGCAGC 1200

TTATAA

1206

## 5 (2) INFORMATION FOR SEQUENCE ID NO: 88:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1083
- 10 (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : cDNA (OCIF-DCR1)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 88:

20 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAACCTT GCCCTGACCA CTACTACACA GACAGCTGGC ACACCACTGA CGAGTGTCTA 120  
 TACTGCAGCC CCGTGTGCAA GGAGCTGCAG TACGTCAAGC AGGAGTGCAA TCGCACCCAC 180  
 AACCCGTGT GCGAATGCAA GGAAGGGCGC TACCTTGAGA TAGAGTTCTG CTTGAAACAT 240  
 AGGAGCTGCC CTCTGGATT TGGAGTGGTG CAAGCTGGAA CCCCAGAGCG AAATACAGTT 300  
 25 TGCAAAAGAT GTCCAGATGG GTTCTTCTCA AATGAGACGT CATCTAAAGC ACCCTGTAGA 360  
 AACACACAA ATTGCAGTGT CTTGGTCTC CTGCTAACTC AGAAAGGAAA TGCAACACAC 420  
 GACAACATAT GTTCCGGAAA CAGTGAATCA ACTCAAAAAT GTGGAATAGA TGTTACCTG 480  
 30 TGTGAGGAGG CATTCTTCAG GTTTGCTGTT CCTACAAAGT TTACGCCTAA CTGGCTTAGT 540  
 GTCTTGGTAG ACAATTGCCC TGGCACCAAA GTAAACGCAG AGAGTGTAGA GAGGATAAAA 600  
 CGGCAACACA GCTCACAAGA ACAGACTTTC CAGCTGCTGA AGTTATGGAA ACATCAAAC 660  
 35 AAAGACCAAG ATATAGTCAA GAAGATCATC CAAGATATTG ACCTCTGTGA AAACAGCGTG 720  
 CAGCGGCACA TTGGACATGC TAACCTCACC TTGAGGAGC TTGCTAGCTT GATGGAAAGC 780  
 TTACCGGGAA AGAAAGTGGG AGCAGAAGAC ATTGAAAAAA CAATAAAGGC ATGCAAACCC 840  
 40 AGTGACCAGA TCCTGAAGCT GCTCAGTTG TGGCGAATAA AAAATGGCGA CCAAGACACC 900  
 TTGAAGGGCC TAATGCACGC ACTAAAGCAC TCAAAAGACGT ACCACTTCC CAAAAGTGTG 960  
 ACTCAGAGTC TAAAGAAGAC CATCAGGTTG CTTCACAGCT TCACAATGTAA CAAATTGTAT 1020  
 CAGAAGTTAT TTTAGAAAT GATAGGTAAC CAGGTCCAAT CAGTAAAAAT AAGCTGCTTA 1080  
 TAA

1083

## 45 (2) INFORMATION FOR SEQUENCE ID NO: 89:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1080
- 50 (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR2)

5 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 89:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
10 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
20 GTGTGCGCCG AATGCAAGGA AGGGCGCTAC CTTGAGATAG AGTTCTGCTT GAAACATAGG 240  
AGCTGCCCTC CTGGATTGG AGTGGTGAA GCTGGAACCC CAGAGCGAAA TACAGTTGC 300  
15 AAAAGATGTC CAGATGGTT CTTCTCAAAT GAGACGTCACT CAAAGCACC CTGTAGAAAA 360  
CACACAAATT GCAGTGTCTT TGGTCTCCTG CTAACTCAGA AAGGAAATGC AACACACGAC 420  
AACATATGTT CCGGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCTGTGT 480  
25 GAGGAGGCAT TCTTCAGGTT TGCTGTTCT ACAAAAGTTA CGCCTAATG GCTTAGTGTC 540  
TTGGTAGACA ATTTGCTGG CACCAAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600  
CAACACAGCT CACAAGAACAA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660  
GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTGAAAA CAGCGTGCAG 720  
20 CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780  
CCGGGAAAGA AAGTGGGAGC AGAAGACATT GAAAAAAACAA TAAAGGCATG CAAACCCAGT 840  
GACCAGATCC TGAAGCTGCT CAGTTTGTGG CGAATAAAAAA ATGGCGACCA AGACACCTTG 900  
30 AAGGGCCTAA TGCACGCACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCACT 960  
CAGAGTCTAA AGAAGACCAT CAGGTTCTT CACAGCTTC CAATGTACAA ATTGTATCAG 1020  
AAGTTATTTT TAGAAATGAT AGGTAACCAAG GTCCAATCAG TAAAATAAG CTGCTTATAA 1080

35 (2) INFORMATION FOR SEQUENCE ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 1092

40 (B) TYPE : nucleic acid

(C) STRANDEDNESS : single

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR3)

45 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 90:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
50 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCG CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACCGAGTGT 240

5 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCAGATG TCCAGATGGG TTCTTCTCAA ATGAGACGTC ATCTAAAGCA 360  
 CCCTGTAGAA AACACACAAA TTGCAGTGTG TTTGGTCTCC TGCTAACTCA GAAAGGAAAT 420  
 GCAACACACG ACAACATATG TTCCGGAAAC AGTGAATCAA CTCAAAAATG TGGAATAGAT 480  
 GTTACCCCTGT GTGAGGAGGC ATTCTTCAGG TTTGCTGTTC CTACAAAGTT TACGCCAAC 540  
 10 TGGCTTAGTG TCTTGGTAGA CAATTTGCCT GGCACCAAAG TAAACGAGA GAGTGTAGAG 600  
 AGGATAAAAC GGCAACACAG CTCACAAGAA CAGACTTTCC AGCTGCTGAA GTTATGGAAA 660  
 CATCAAAACA AAGACCAAGA TATAGTCAG AAGATCATCC AAGATATTGA CCTCTGTGAA 720  
 15 AACAGCGTGC AGCGGCACAT TGGACATGCT AACCTCACCT TCGAGCAGCT TCGTAGCTTG 780  
 ATGGAAAGCT TACCGGGAAA GAAAGTGGGA GCAGAAGACA TTGAAAAAAC AATAAAGGCA 840  
 TGCAAAACCA GTGACCAGAT CCTGAAGCTG CTCAGTTGT GGCAGATAAA AAATGGCGAC 900  
 CAAGACACCT TGAAGGGCCT AATGCACGCA CTAAAGCACT CAAAGACGTA CCACTTCCC 960  
 20 AAAACTGTCA CTCAGAGTCT AAAGAAGACC ATCAGGTTCC TTCACAGCTT CACAATGTAC 1020  
 AAATTGTATC AGAAGTTATT TTTAGAAATG ATAGGTAACC AGGTCCAATC AGTAAAAATA 1080  
 AGCTGCTTAT AA 1092

25 (2) INFORMATION FOR SEQUENCE ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1080
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DCR4)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 91:

30 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTG 120  
 40 TGTGACAAAT GTCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACCGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 45 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGCAAAT CGCGAAACAG TGAATCAACT CAAAAATGTG GAATAGATGT TACCCGTG 480  
 GAGGAGGCAT TCTTCAGGTT TGCTGTTCT ACAAAAGTTA CGCCTAACTG GCTTAGTGTC 540  
 50 TTGGTAGACA ATTGCCCTGG CACCAAGTA AACGCAGAGA GTGTAGAGAG GATAAAACGG 600  
 CAACACAGCT CACAAGAACAA GACTTTCCAG CTGCTGAAGT TATGGAAACA TCAAAACAAA 660

5 GACCAAGATA TAGTCAAGAA GATCATCCAA GATATTGACC TCTGTGAAAA CAGCGTGCAG 720  
 CGGCACATTG GACATGCTAA CCTCACCTTC GAGCAGCTTC GTAGCTTGAT GGAAAGCTTA 780  
 CCGGGAAAGA AAGTGGGAGC AGAACACATT GAAAAAAACAA TAAAGGCATG CAAACCCAGT 840  
 GACCAGATCC TGAAGCTGCT CAGTTTGAG CGAATAAAAA ATGGCGACCA AGACACCTTG 900  
 10 AAGGGCCTAA TGCACCGACT AAAGCACTCA AAGACGTACC ACTTTCCCAA AACTGTCACT 960  
 CAGAGTCTAA AGAAGACCAT CAGGTTCCCTT CACAGCTTCA CAATGTACAA ATTGTATCAG 1020  
 AAGTTATTT TAGAAATGAT AGGTAACCAG GTCCAATCAG TAAAAATAAG CTGCTTATAA 1080

## (2) INFORMATION FOR SEQUENCE ID NO: 92:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 981
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

## (ii) MOLECULE TYPE : cDNA (OCIF-DDD1)

## (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 92:

25 ATGAACAAC TGTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AACCTCTCA TCAGCTGTG 120  
 30 TGTGACAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGGATG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 35 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTTGAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
 AGAAAACACA CAAATTGCAAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 40 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATATTGAC 600  
 CTCTGTGAAA ACAGCGTGCA GCGGCACATT GGACATGCTA ACCTCACCTT CGAGCAGCTT 660  
 CGTAGCTTGA TGGAAAGCTT ACCGGGAAAG AAAGTGGGAG CAGAAGACAT TGAAAAAAACA 720  
 45 ATAAAGGCAT GCAAACCCAG TGACCAAGATC CTGAAGCTGC TCAGTTGTG GCGAATAAAA 780  
 AATGGCGACC AAGACACCTT GAAGGGCCTA ATGCACGCAC TAAAGCACTC AAAGACGTAC 840  
 CACTTCCCA AAACTGTCAAC TCAGAGTCTA AAGAAGACCA TCAGGTTCCCT TCACAGCTTC 900  
 ACAATGTACA AATTGTATCA GAAGTTATTT TTAGAAATGA TAGGTAACCA GGTCCAATCA 960  
 50 GTAAAAATAA GCTGCTTATA A 981

## (2) INFORMATION FOR SEQUENCE ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 984
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-DDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 93:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCCGACC 300  
CACAAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
AGAAAACACA CAAATTGCAAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAAA AATGTGGAAT AGATGTTACC 600  
CTGCTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTACAA AGTTTACGCC TAACTGGCTT 660  
AGTGTCTTGG TAGACAATTG GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGACG CACTAAAGCA CTCAAAGACG 840  
TACCACTTTC CCAAAACTGT CACTCAGAGT CTAAGAAGA CCATCAGGTT CCTTCACAGC 900  
TTCACAATGT ACAAAATTGTA TCAGAAGTTA TTTTAGAAA TGATAGGTAA CCAGGTCCAA 960  
TCAGTAAAAA TAAGCTGCTT ATAA 984

(2) INFORMATION FOR SEQUENCE ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 1200
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CL)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 94:

ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60

5 CAGGAAACGT TTCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCTCCTGG TACCTACCTA AAACAACACT GTACAGAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 10 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
 GTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
 CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
 15 CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCTTACAA AGTTACGCC TAACTGGCTT 660  
 AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACCG CAGAGAGTGT AGAGAGGATA 720  
 AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
 20 AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
 GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
 AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAAA 960  
 CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAATGG CGACCAAGAC 1020  
 25 ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTCAAAGA CGTACCACTT TCCCAAAACT 1080  
 GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCTTCACA GCTTCACAAT GTACAAATTG 1140  
 TATCAGAAGT TATTTTTAGA AATGATAGGT ACCAGGTCC AATCAGTAAA AATAAGCTAA 1200

30 (2) INFORMATION FOR SEQUENCE ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH : 1056
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

40 (ii) MOLECULE TYPE : cDNA (OCIF-CC)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 95:

45 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTCTGGACA TCTCCATTAA GTGGACCACC 60  
 CAGGAAACGT TTCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
 TGTGACAAAT GTCTCCTGG TACCTACCTA AAACAACACT GTACAGAAA GTGGAAGACC 180  
 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
 50 CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
 CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420

GTGGCAAAA GATGCCAGA TGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCGTCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
CTGTGTGAGG AGGCATTCTT CAGGTTTGCCT GTTCCTACAA AGTTTACGCC TAACTGGCTT 660  
AGTGTCTTGG TAGACAATTG GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCTGTAG CTTGATGGAA 900  
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCGATGCAAA 960  
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCGAA TAAAAAAATGG CGACCAAGAC 1020  
ACCTTGAAGG GCCTAATGCA CGCACTAAAG CACTGAA 1056

(2) INFORMATION FOR SEQUENCE ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 819
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CDD2)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 96:

ATGAAACACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGACACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
GTTTGCAAAA GATGCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
AGAAAACACA CAAATTGCAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTCGGG AAACAGTGAA TCAACTCAA AATGTTGAAT AGATGTTACC 600  
CTGTGTGAGG AGGCATTCTT CAGGTTGCT GTTCCCTACAA AGTTACGCC TAACTGGCTT 660  
AGTGTCTTGG TAGACAATT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
AACAAAGACC AAGATATAGT CAAGAACAGTC ATCCAATGA 819

(2) INFORMATION FOR SEQUENCE ID NO: 87:

(2) INFORMATION FOR SEQUENCE ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 321
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- 10 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CCR3)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 99:

15 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
20 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAAACCGCG TGTGCGAATG A 321

25 (2) INFORMATION FOR SEQUENCE ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH : 1182
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CBst)

35 (xi) SEQUENCE DESCRIPTION :SEQ ID NO: 100:

40 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
45 CACAAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
50 AGAAAACACA CAAATTGCAG TGTCTTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCCTACAA AGTTTACGCC TAACTGGCTT 660

AGTGTCTTGG TAGACAATTT GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
AACAAAGACC AAGATATAGT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
GTGCAGCGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCATGCAA 960  
CCCAGTGACC AGATCCTGAA GCTGCTCAGT TTGTGGCAGA TAAAAAAATGG CGACCAAGAC 1020  
ACCTTGAAGG GCCTAAATGCA CGCACTAAAG CACTCAAAGA CGTACCAACTT TCCCAAAACT 1080  
GTCACTCAGA GTCTAAAGAA GACCATCAGG TTCCCTTCACA GCTTCACAAT GTACAAATTG 1140  
TATCAGAAAGT TATTTTTAGA AATGATAGGT AACCTAGTCT AG 1182

(2) INFORMATION FOR SEQUENCE ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH : 966
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CSph)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 101:

ATGAAACAAT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCCCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGACACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCCGACC 300  
CACAAACCGGG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCCTGT 480  
AGAAAACACA CAAATTGCAAG TGTCTTGGT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AAACAGTGAA TCAACTCAA AATGTGGAAT AGATGTTACC 600  
CTGTGTGAGG AGGCATTCTT CAGGTTTGCT GTTCCCTACAA AGTTACGCC TAACTGGCTT 660  
AGTGTCTTGG TAGACAATTG GCCTGGCACC AAAGTAAACG CAGAGAGTGT AGAGAGGATA 720  
AAACGGCAAC ACAGCTCACA AGAACAGACT TTCCAGCTGC TGAAGTTATG GAAACATCAA 780  
AACAAAGACC AAGATATACT CAAGAAGATC ATCCAAGATA TTGACCTCTG TGAAAACAGC 840  
GTGCAGGGC ACATTGGACA TGCTAACCTC ACCTTCGAGC AGCTTCGTAG CTTGATGGAA 900  
AGCTTACCGG GAAAGAAAGT GGGAGCAGAA GACATTGAAA AAACAATAAA GGCTAGTCTA 960  
GACTAG 966

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 594  
(B) TYPE : nucleic acid  
(C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
10 (ii) MOLECULE TYPE : cDNA (OCIF-CDD1)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 97:

15 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCTCTAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCTCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
20 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
25 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
AGAAAACACA CAAATTGCAG TGTCTTGGT CTCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAACA TATGTTCCGG AACAGTGAA TCAACTCAA AATGTGGAAT ATGA. 594

30 (2) INFORMATION FOR SEQUENCE ID NO: 98:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH : 432  
(B) TYPE : nucleic acid  
35 (C) STRANDEDNESS : single  
(D) TOPOLOGY : linear  
(ii) MOLECULE TYPE : cDNA (OCIF-CCR4)  
(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 98:

40 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCTCTAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
TGTGACAAAT GTCTCTCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
45 GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCCAG TGACGAGTGT 240  
CTATACTGCA GCCCCGTGTG CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
50 CATAGGAGCT GCCCTCTGG ATTTGGAGTG GTGCAAGCTG GAACCCAGA GCGAAATACA 420  
GTTTGCAAAAT GA 432

(2) INFORMATION FOR SEQUENCE ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH : 564
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- 10 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-CBsp)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 102:

15 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
20 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGGAGTGT 240  
CTATACTGCA GCCCCGTGTC CAAGGAGCTG CAGTACGTCA AGCAGGAGTG CAATCGCACC 300  
25 CACAACCGCG TGTGCGAATG CAAGGAAGGG CGCTACCTTG AGATAGAGTT CTGCTTGAAA 360  
CATAGGAGCT GCCCTCCTGG ATTTGGAGTG GTGCAAGCTG GAACCCCAGA GCGAAATACA 420  
30 GTTTGCAAAA GATGTCCAGA TGGGTTCTTC TCAAATGAGA CGTCATCTAA AGCACCCGT 480  
AGAAAACACA CAAATTGCAG TGTCTTGTT CTCCCTGCTAA CTCAGAAAGG AAATGCAACA 540  
CACGACAAACA TATGTTCCGG CTAG 564

(2) INFORMATION FOR SEQUENCE ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH : 255
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : single
- 40 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA (OCIF-Pst)

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 103:

45 ATGAACAACT TGCTGTGCTG CGCGCTCGTG TTTCTGGACA TCTCCATTAA GTGGACCACC 60  
CAGGAAACGT TTCCCTCCAAA GTACCTTCAT TATGACGAAG AAACCTCTCA TCAGCTGTTG 120  
50 TGTGACAAAT GTCCCTCCTGG TACCTACCTA AAACAACACT GTACAGCAAA GTGGAAGACC 180  
GTGTGCGCCC CTTGCCCTGA CCACTACTAC ACAGACAGCT GGCACACCAAG TGACGGAGTGT 240  
CTATACCTAG TCTAG 255

## (2) INFORMATION FOR SEQUENCE ID NO: 104:

## (i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH : 1317  
 (B) TYPE : nucleic acid  
 (C) STRANDEDNESS : double  
 10 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : human OCIF genomic DNA-1

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 104:

15	CTGGAGACAT ATAACTGAA CACTTGGCCC TGATGGGAA GCAGCTCTGC AGGGACTTT	60
	TCAGCCATCT GTAAACAATT TCAGTGGCAA CCCCGAAGT GTAATCCATG AATGGGACCA	120
	CACTTTACAA GTCATCAAGT CTAACCTCTA GACCAGGGAA TTAATGGGG AGACAGCGAA	180
20	CCCTAGAGCA AAGTGCAAA CTTCTGTCGA TAGCTTGAGG CTAGTGAAA GACCTCGAGG	240
	AGGCTACTCC AGAAGTTCAG CGCGTAGGAA GCTCCGATAC CAATAGCCCT TTGATGATGG	300
	TGGGGTTGGT GAAGGGAACA GTGCCCGCA AGGTTATCCG TGCCCCAGGC AGTCCAATT	360
25	TCACTCTGCA GATTCTCTCT GGCTCTAACT ACCCCAGATA ACAAGGAGTG AATGCAGAAT	420
	AGCACGGGCT TTAGGGCCAA TCAGACATTA GTTAGAAAAA TTCTACTAC ATGGTTTATG	480
	TAAACTTGAA GATGAATGAT TGCGAACTCC CCGAAAAGGG CTCAGACAAT GCCATGCATA	540
	AAGAGGGGCC CTGTAATTG AGGTTTCAGA ACCCGAAGTG AAGGGTCAG GCAGCCGGT	600
30	ACGGCGGAAA CTCACAGCTT TCGCCCAGCG AGAGGACAAA GGTCTGGAC ACACTCCAAC	660
	TGGTCCCGGA TCTTGGCTGG ATCGGACTCT CAGGGTGGAG GAGACACAAG CACAGCAGCT	720
	GCCCAGCGTG TGCCCAGCCC TCCCACCGCT GGTCCCGGCT GCCAGGAGGC TGGCCGCTGG	780
35	CGGGAAGGGG CGGGGAAACC TCAGAGCCCC GCGGAGACAG CAGCCGCCTT GTTCTCAGC	840
	CCGGTGGCTT TTTTTCCCC TGCTCTCCA GGGGACAGAC ACCACCGCCC CACCCCTCAC	900
	GCCCCACCTC CCTGGGGGAT CCTTCCCGCC CCAGCCCTGA AAGCGTTAAT CCTGGAGCTT	960
40	TCTGCACACC CCCCCACCGC TCCCGCCAA GCTTCCTAAA AAAGAAAGGT GCAAAGTTG	1020
	GTCCAGGATA GAAAATGAC TGATCAAAGG CAGGCATAAC TTCTGTTGC CGGGACGCTA	1080
	TATATAACGT GATGAGCGCA CGGGCTGCGG AGACGCACCG GAGCGCTCGC CCAGCCGCCG	1140
	CCTCCAAGCC CCTGAGGTTT CGGGGACCA CA ATG AAC AAG TTG CTG TGC TGC	1193

Met Asn Lys Leu Leu Cys Cys

-20

-15

45	GCG CTC GTG GTAAAGTCCCT GGGCCAGCCG ACGGGTGCCG GGCGCCTGGG	1242
	Ala Leu Val	

50	GAGGCTGCTG CCACCTGGTC TCCCAACCTC CCAGCGGACC GGCGGGAAA AAGGCTCCAC	1302
----	--	------

TCGCTCCCTC CCAAG

1317

5 (2) INFORMATION FOR SEQUENCE ID NO: 105:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH :
- (B) TYPE : nucleic acid
- (C) STRANDEDNESS : double
- (D) TOPOLOGY : linear

15 (ii) MOLECULE TYPE : human OCIF genomic DNA-2

(xi) SEQUENCE DESCRIPTION :SEQ ID NO: 105:

GCTTACTTTC TGCCAAATCT CATTAGGCTT AAGGTAATAC AGGACTTGA GTCAAATGAT @60  
 ACTGTTGCAC ATAAGAACAA ACCTATTTTC ATGCTAAGAT GATGCCACTG TGTTCCCTTC 120  
 TCCTTCTAG TTT CTG GAC ATC TCC ATT AAG TGG ACC ACC CAG GAA ACG TTT 171  
 Phe Leu Asp Ile Ser Ile Lys Trp Thr Thr Gln Glu Thr Phe  
 -10 -5 -1 1  
  
 CCT CCA AAG TAC CTT CAT TAT GAC GAA GAA ACC TCT CAT CAG CTG TTG 219  
 Pro Pro Lys Tyr Leu His Tyr Asp Glu Glu Thr Ser His Gln Leu Leu  
 5 10 15  
  
 TGT GAC AAA TGT CCT CCT GGT ACC TAC CTA AAA CAA CAC TGT ACA GCA 267  
 Cys Asp Lys Cys Pro Pro Gly Thr Tyr Leu Lys Gln His Cys Thr Ala  
 20 25 30 35  
  
 AAG TGG AAG ACC GTG TGC GCC CCT TGC CCT GAC CAC TAC TAC ACA GAC 315  
 Lys Trp Lys Thr Val Cys Ala Pro Cys Pro Asp His Tyr Tyr Thr Asp  
 40 45 50  
  
 AGC TGG CAC ACC AGT GAC GAG TGT CTA TAC TGC AGC CCC GTG TGC AAG 363  
 Ser Trp His Thr Ser Asp Glu Cys Leu Tyr Cys Ser Pro Val Cys Lys  
 55 60 65  
  
 GAG CTG CAG TAC GTC AAG CAG GAG TGC AAT CGC ACC CAC AAC CGC GTG 411  
 Glu Leu Gln Tyr Val Lys Gln Glu Cys Asn Arg Thr His Asn Arg Val  
 70 75 80

5	TGC GAA TGC AAG GAA GGG CGC TAC CTT GAG ATA GAG TTC TGC TTG AAA Cys Glu Cys Lys Glu Gly Arg Tyr Leu Glu Ile Glu Phe Cys Leu Lys 85	90	95	459
10	CAT AGG AGC TGC CCT CCT GGA TTT GGA GTG GTG CAA GCT G GTACGTGTCA His Arg Ser Cys Pro Pro Gly Phe Gly Val Val Gln Ala 100	105	110	509
15	ATGTGCAGCA AAATTAATTAA GGATCATGCA AAGTCAGATA GTTGTGACAG TTTAGGAGAA CACTTTTGTGTT CTGATGACAT TATAGGATAG CAAATTGCAA AGGTAAATGAA ACCTGCCAGG TAGGTACTAT GTGTCTGGAG TGCTTCCAAA GGACCAATTGC TCAGAGGAAT ACTTTGCCAC TACAGGGCAA TTTAATGACA AATCTCAAAT GCAGCAAATT ATTCTCTCAT GAGATGCATG ATGGTTTTTTT TTTTTTTTTT TAAAGAAACA AACTCAAGTT GCACTATTGA TAGTTGATCT 20			569
25	ATACCTCTAT ATTTCACTTC AGCATGGACA CCTTCAAACT GCAGCACTTT TTGACAAACA TCAGAAATGT TAATTTATAC CAAGAGAGTA ATTATGCTA TATTAATGAG ACTCTGGAGT GCTAACAAATA AGCAGTTATA ATTAATTATG TAAAAAATGA GAATGGTGAG GGGATTGCA TTTCATTATT AAAACAAGG CTAGTTCTTC CTTTAGCATG GGAGCTGAGT GTTGGGAGG GTAAGGACTA TAGCAGAAC TCTTCAATGA GCTTATTCTT TATCTTAGAC AAAACAGATT 30			629
35	GTCAAGCCAA GAGCAAGCAC TTGCTATAA ACCAAGTGTCTTCTTCTTGTG CATTGAAAC AGCATTGGTC AGGGCTCATG TGTATTGAAT CTTTAAACC AGTAACCCAC GTTTTTTTTC TGCCACATTT GCGAAGCTTC AGTGCAGCCT ATAACCTTTC ATAGCTTGAG AAAATTAAGA GTATCCACTT ACTTAGATGG AAGAAGTAAT CAGTATAGAT TCTGATGACT CAGTTGAG 40			689
45	CAGTGTCTCT CAACTGAAGC CCTGCTGATA TTTTAAGAAA TATCTGGATT CCTAGGCTGG ACTCCTTTTT GTGGGCAGCT GTCCCTGCGCA TTGAGAATT TTGGCAGCAC CCCTGGACTC TAGCCACTAG ATACCAATAG CAGTCCTTCC CCCATGTGAC AGCCAAAAAT GTCTTCAGAC ACTGTCAAAT GTGCCAGGT GGCAAAATCA CTCCCTGGTTG AGAACAGGGT CATCAATGCT 50			749
55	AAGTATCTGT AACTATTITA ACTCTAAAAA CTGAGATAT ACAAAAGTCTA AATTATTAGA CGACCAATAC TTAGGTTTA AAGGCATACA AATGAAACAT TCAAAATCA AAATCTATTG TGTCTCAA ATAGTGAATC TTATAAAATT AATCACAGAA GATGCAAATT GCATCAGAGT CCCTTAAAT TCCTCTTCGT ATGAGTATTG GAGGGAGGAA TTGGTGATAG TTCTACTTT CTATTGGATG GTACTTTGAG ACTCAAAAGC TAAGCTAAGT TGTGTGTGTG TCAGGGTGG GGGTGTGGAA TCCCATCAGA TAAAAGCAA TCCATGTAAT TCATTGAGTA AGTTGTATAT GTAGAAAAAT GAAAAGTGGG CTATGCAGCT TGGAAACTAG AGAATTGAA AAAATAATGG AAATCACAAG GATCTTCTT AAATAAGTAA GAAAATCTGT TTGAGAATG AAGCAAGCAG GCAGCCAGAA GACTCAGAAC AAAAGTACAC ATTTTACTCT GTGTACACTG GCAGCACAGT GGGATTATT TACCTCTCCC TCCCTAAAAA CCCACACAGC GGTTCTCTT GGGAAATAAG 2129			809
				2189

	AGGTTTCCAG CCCAAAGAGA AGGAAAGACT ATGTGGTGT ACTCTAAAAA GTATTTAATA	2249
5	ACCGTTTGT TGTTGCTGTT GCTGTTTGA AATCAGATTG TCTCCTCTCC ATATTTTATT	2309
	TAACCTCATTG TGTTAATTCC TGTGGAATTA CTTAGAGCAA GCATGGTGAA TTCTCAACTG	2369
	TAAAGCCAAA TTTCTCCATC ATTATAATT CACATTTGC CTGGCAGGTT ATAATTTTA	2429
	TATTTCCACT GATAGTAATA AGGTAAAATC ATTACTAGA TGGATAGATC TTTTCATAA	2489
10	AAAGTACCAT CAGTTATAGA GGGAAAGTCAT GTTCATGTT AGGAAGGTCA TTAGATAAAG	2549
	CTTCTGAATA TATTATGAAA CATTAGTTCT GTCAATTCTTA GATTCTTTT GTAAATAAC	2609
	TTTAAAAGCT AACTTACCTA AAAGAAATAT CTGACACATA TGAACCTCTC ATTAGGATGC	2669
15	AGGAGAAAGAC CCAAGCCACA GATATGTATC TGAAGAATGA ACAAGATTCT TAGGCCCGC	2729
	ACGGTGGCTC ACATCTGTAA TCTCAAGAGT TTGAGAGGTC AAGGCGGGCA GATCACCTGA	2789
	GGTCAGGAGT TCAAGACCAG CCTGGCCAAC ATGATGAAAC CCTGCCCTA CTAAAAATAC	2849
	AAAAATTAGC AGGGCATGGT GGTGCATGCC TGCAACCTA GCTACTCAGG AGGCTGAGAC	2909
20	AGGAGAAATCT CTTGAACCTCT CGAGGCGGAG GTTGTGGTGA GCTGAGATCC CTCTACTGCA	2969
	CTCCAGCCTG GGTGACAGAG ATGAGACTCC GTCCCTGCCG CCGCCCCCGC CTTCCCCCCC	3029
	AAAAAGATTG AGGAGATTC TTCTTCATGC AGAACATAAG GCAGTCAACA AAGGGAGACC TGGGTCCAGG	3089
	TGTCCAAGTC ACTTATTTCG AGTAAATTAG CAATGAAAGA ATGCCATGGA ATCCCTGCC	3149
25	AAATACCTCT GCTTATGATA TTGTAGAATT TGATATAGAG TTGTATCCCA TTAAAGGAGT	3209
	AGGATGTAGT AGGAAAGTAC TAAAAACAAA CACACAAACA GAAAACCCCTC TTTGCTTTGT	3269
	AAGGTGGTTC CTAAGATAAT GTCACTGCAA TGCTGGAAAT AATATTTAAT ATGTGAAGGT	3329
30	TTAGGCTGT GTTTCCCT CCTGTTCTT TTTCTGCCA GCCCTTGTC ATTTTGCGAG	3389
	GTCAATGAAT CATGTAGAAA GAGACAGGAG ATGAAACTAG AACCAGTCCA TTTGCCCT	3449
	TTTTTATTT TCTGGTTTG GTAAAAGATA CAATGAGGTAA GGAGGTTGAG ATTTATAAAT	3509
	GAAGTTAAT AAGTTCTGT AGCTTGATT TTTCTTTTC ATATTTGTTA TCTTGATAA	3569
35	GCCAGAATTG GCCTGTAAGA TCTACATATG GATATTGAAG TCTAAATCTG TTCAACTAGC	3629
	TTACACTAGA TGGAGATATT TTCATATTCA GATACACTGG AATGTATGAT CTAGCCATGC	3689
	GTAATATAGT CAAGTGGTTG AAGGTATTAA TTTTAATAG CGTCTTTAGT TGTGGACTGG	3749
40	TTCAAGTTT ITCGCCATG ATTTCTCAA ATTTATCAA TATTTTCCA TCATGAAGTA	3809
	AAATGCCCTT GCAGTCACCC TTCTGAAGT TTGAACGACT CTGCTGTTT AAACAGTTA	3869
	AGCAAATGGT ATATCATCTT CCGTTTACTA TGAGCTTAA CTGCAGGCTT ACGTTTGA	3929
	GTCAGCGGCC AACTTTATTG CCACCTCAA AAGTTTATA TAATGTTGTA AATTTTACT	3989
45	TCTCAAGGTT AGCATACTTA GGAGTTGCTT CACAATTAGG ATTCAAGGAAA GAAAGAACTT	4049
	CAGTAGGAAC TGATTGGAAT TTAATGATGC AGCATTCAAT GGGTACTAAT TTCAAAGAAT	4109
	GATATTACAG CAGACACACA GCAGTTATCT TGATTTCTA GGAATAATTG TATGAAGAAT	4169
	ATGGCTGACA ACACGGCCTT ACTGCCACTC AGCGGAGGCT GGACTAATGA ACACCCCTACC	4229
50	CTTCTTCTCCT TTCTCTCAC ATTTCATGAG CGTTTGTAG GTAACCGAGAA AATTGACTTG	4289
	CATTTGCATT ACAAGGAGGA GAAACTGGCA AAGGGGATGA TGGTGGAAAGT TTTGTTCTGT	4349

5	CTAATGAAGT GAAAAATGAA AATGCTAGAG TTTTGTCAA CATAATAGTA CCAGTAAAAA CCAAGTGAAA AGCTTTCCA AAACGTGTT AAGAGGGCAT CTGCTGGAA ACCATTTGAG GAGAAGGTAC TAAATTGCTT GGTATTTCC GTAG GA ACC CCA GAG CGA AAT ACA	4409 4469 4523
	Gly Thr Pro Glu Arg Asn Thr	
	115	
10	GTT TGC AAA AGA TGT CCA GAT GGG TTC TTC TCA AAT GAG ACG TCA TCT Val Cys Lys Arg Cys Pro Asp Gly Phe Phe Ser Asn Glu Thr Ser Ser	4571
	120 125 130 135	
15	AAA GCA CCC TGT AGA AAA CAC ACA AAT TGC AGT GTC TTT GGT CTC CTG Lys Ala Pro Cys Arg Lys His Thr Asn Cys Ser Val Phe Gly Leu Leu	4619
	140 145 150	
20	CTA ACT CAG AAA GGA AAT GCA ACA CAC GAC AAC ATA TGT TCC GGA AAC Leu Thr Gln Lys Gly Asn Ala Thr His Asp Asn Ile Cys Ser Gly Asn	4667
	155 160 165	
25	AGT GAA TCA ACT CAA AAA TGT GGA ATA G GTAATTACAT TCCAAAATAC Ser Glu Ser Thr Gln Lys Cys Gly Ile	4715
	170 175	
30	GTCTTGTAC GATTTGTAG TATCATCTCT CTCTCTGAGT TGAACACAAG GCCTCCAGCC ACATTCCTGG TCAAACCTAC ATTTCCCTT TCTTGAATCT TAACCAGCTA AGGCTACTCT	4775 4835
	CGATGCATTA CTGCTAAAGC TACCACTCAG AATCTCTCAA AAACTCATCT TCTCACAGAT	4895
35	AACACCTCAA AGCTTGATTT TCTCTCCTT CACACTGAAA TCAAATCTTG CCCATAGGCA AAGGGCAGTG TCAAGTTTG CACTGAGATG AAATTAGGAG AGTCCAAACT GTAGAATTCA	4955 5015
	CGTTGTGT TATTACTTTC ACGAATGTCT GTATTATTAA CTAAAGTATA TATTGGCAAC	5075
40	TAAGAAGCAA AGTGATATAA ACATGATGAC AAATTAGGCC AGGCATGGTG GCTTACTCCT	5135
	ATAATCCCAA CATTGGGG GGCCAAGGTA GGCAAGATCAC TTGAGGTCAAG GATTCAAGA	5195
45	CCAGCCTGAC CAACATGGTG AAACCTTGTCT TCTACTAAAA ATACAAAAT TAGCTGGCA TGGTAGCAGG CACTTCTAGT ACCAGCTACT CAGGGCTGAG GCAGGAGAAT CGCTTGAACC	5255 5315
	CAGGAGATGG AGGTTGCAGT GAGCTGAGAT TGTACCACTG CACTCCAGTC TGGGCAACAG	5375
50	AGCAAGATT CATCACACAC ACACACACAC ACACACACAC ACACATTAGA AATGTGTACT	5435
	TGGCTTGT TACCTATGGTA TTAGTGCATC TATTGCATGG AACTCCAAG CTACTCTGGT	5495
	TGTGTTAACG TCTTCATTGG GTACAGGTCA CTAGTATTAA GTTCAGGTAA TTCGGATGCA	5555

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5	TTCCACGGTA GTGATGACAA TTCATCAGGC TAGTGTGTGT GTTCACCTTG TCACTCCAC	5615		
	CACTAGACTA ATCTCAGACC TTCACTAAA GACACATTAC ACTAAAGATG ATTTGCTTT	5675		
	TTGTGTTAA TCAAGCAATG GTATAAACCA GCTTGAATCT CCCCAAACAG TTTTCGTAC	5735		
	TACAAAGAAG TTTATGAAGC AGAGAAATGT GAATTGATAT ATATATGAGA TTCTAACCA	5795		
	GTTCCAGCAT TGTTCATTG TGTAAATTGAA ATCATAGACA AGCCATTITA GCCTTTGCTT	5855		
10	TCTTATCTAA AAAAAAAA AAAAAATGA AGGAAGGGT ATTAAAAGGA GTGATCAAAT	5915		
	TTAACATTTC TCTTTAATT ATTCACTTTT AATTCTACTT TTTCATTT ATTGTGCACT	5975		
	TAATATGTGG TACTGTGCTA TAGAGGCTTT AACATTATA AAAACACTGT GAAAGTTGCT	6035		
	TCAGATGAAT ATAGGTAGTA GAACGGCAGA ACTAGTATT AAAGCCAGGT CTGATGAATC	6095		
15	CAAAAACAAA CACCCATTAC TCCCATTTC TGGGACATAC TTACTCTACC CAGATGCTCT	6155		
	GGGCTTGTA ATGCCTATGT AAATAACATA GTTTATGTT TGGTTATTTT CCTATGTAAT	6215		
	GTCTACTTAT ATATCTGTAT CTATCTCTTG CTTTGTTC CAAAGGTAAC TATGTGTCTA	6275		
20	AATGTGGGCA AAAATAACA CACTATTCCA AATTACTGTT CAAATTCCTT TAAGTCAGTG	6335		
	ATAATTATTT GTTTGACAT TAATCATGAA GTTCCCTGTG GGTACTAGGT AAACCTTTAA	6395		
	TAGAATGTTA ATGTTTGTAT TCATTATAAG AATTTTGGC TGTACTTAT TTACAACAAT	6455		
	ATTTCACTCT AATTAGACAT TTACTAAACT TTCTCTGAA AACATGCC AAAAAAGAAC	6515		
25	ATTAGAAGAC ACGTAAGCTC AGTTGGTCTC TGCCACTAAG ACCAGCCAAC AGAAGCTTGA	6575		
	TTTTATCAA ACTTTGCATT TTAGCATATT TTATCTTGGAA AAATTCAATT GTGTTGGTT	6635		
	TTTGTTTTIG TTTGTATTGA ATAGACTCTC AGAAATCCAA TTGTTGAGTA AATCTCTGG	6695		
30	TTTTCTAAC CTTCTTTAG AT GTT ACC CTG TGT GAG GAG GCA TTC TTC AGG	6747		
	Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg			
	180	185		
35	TTT GCT GTT CCT ACA AAG TTT ACG CCT AAC TGG CTT AGT GTC TTG GTA	6795		
	Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val			
	190	195	200	
40	GAC AAT TTG CCT GGC ACC AAA GTA AAC GCA GAG AGT GTA GAG AGG ATA	6843		
	Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile			
	205	210	215	
45	AAA CGG CAA CAC AGC TCA CAA GAA CAG ACT TTC CAG CTG CTG AAG TTA	6891		
	Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu			
	220	225	230	235
50	TGG AAA CAT CAA AAC AAA GAC CAA GAT ATA GTC AAG AAG ATC ATC CAA G	6940		

Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln

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GTAAATTACAT TCCAAAATAC GTCTTTGTAC GATTTTGTAG TATCATCTCT CTCTCTGAGT 7000  
 TGAACACAAG GCCTCCAGCC ACATTCTTGG TCAAACATTAC ATTTTCCCTT TCTTGAATCT 7060  
 TAACCAGCTA AGGCTACTCT CGATGCATTA CTGCTAAAGC TACCACTCAG AATCTCTCAA 7120  
 AAACTCATCT TCTCACAGAT AACACCTCAA AGCTTGATT TCTCTCCTT CACACTGAAA 7180  
 TCAAATCTTG CCCATAGGCA AAGGGCAGTG TCAAGTTTGC CACTGAGATG AAATTAGGAG 7240  
 AGTCCAAACT GTAGAATTCA CGTTGTGTGT TATTACTTC ACGAATGTCT GTATTATTAA 7300  
 CTAAAGTATA TATTGGCAAC TAAGAAGCAA AGTGTATATA ACATGTGAC AAATTAGGCC 7360  
 AGGCATGGTG GCTTACTCCT ATAATCCAA CATTGGGG GGCAAGGTA GGCAAGATCAC 7420  
 TTGAGGTCAAG GATTTCAGA CCAGCCTGAC CAACATGGTG AAACCTTGTCT TCTACTAAAA 7480  
 20 ATACAAAAAT TAGCTGGCA TGGTAGCAGG CACTCTAGT ACCAGCTACT CAGGGCTGAG 7540  
 GCAGGAGAAT CGCTTGAACC CAGGAGATGG AGGTTGCAGT GAGCTGAGAT TGTACCACTG 7600  
 CACTCCAGTC TGGGCAACAG AGCAAGATT CATCACACAC ACACACACAC ACACACACAC 7660  
 ACACATTAGA AATGTGTACT TGGCTTTGT ACCTATGGTA TTAGTGCATC TATTGCATGG 7720  
 25 AACTTCCAAG CTACTCTGGT TGTGTAAAGC TCTTCATTGG GTACAGGTCA CTAGTATTAA 7780  
 GTTCAGGTAA TTCCGGATGCA TTCCACGGTA GTGATGACAA TTCACTCAGGC TAGTGTGTGT 7840  
 GTTCACCTTG TCACTCCAC CACTAGACTA ATCTCAGACC TTCACTCAAA GACACATTAC 7900  
 30 ACTAAAGATG ATTTGCTTTT TTGTGTAAAGC TCAAGCAATG GTATAAACCA GCTTGAATCT 7960  
 CCCAAACAG TTTTCGTAAC TACAAAGAAG TTATGAAGC AGAGAAATGT GAATTGATAT 8020  
 ATATATGAGA TTCTAACCCA GTTCCAGCAT TGTTTCATTG TGTAATTGAA ATCATAGACA 8080  
 AGCCATTITA GCCTTTGGTT TCTTATCTAA AAAAAAAA AAAAAATGA AGGAAGGGGT 8140  
 35 ATTTAAAGGA GTGATCAAAT TTAAACATTIC TCTTTAATTAA ATTCAATTTTT AATTTCATT 8200  
 TTTTCATT ATTGTGCACT TACTATGTGG TACTGTGCTA TAGAGGCTTT AACATTATA 8260  
 AAAACACTGT GAAAGTTGCT TCAGATGAAT ATAGGTAGTA GAACGGCAGA ACTAGTATT 8320  
 40 AAAGCCAGGT CTGATGAATC CAAAAACAAA CACCCATTAC TCCCATTTC TGGGACATAC 8380  
 TTACTCTACC CAGATGCTCT GGGCTTGTA ATGCCTATGT AAATAACATA GTTTATGTT 8440  
 TGGTTATTCTT CCTATGTAAT GTCTACTTAT ATATCTGTAT CTATCTTGTCTTGTGTT 8500  
 AAAGGTAAAC TATGTGTCTA AATGTGGCA AAAAATAACA CACTATTCCA AATTACTGTT 8560  
 45 CAAATTCCCTT TAAGTCAGTG ATAATTATTG TTGTTGACAT TAATCATGAA GTTCCCTGTG 8620  
 GGTACTAGGT AAACCTTAA TAGAATGTTA ATGTTTGAT TCATTATAAG AATTTCGGC 8680  
 TGTTACTTAT TTACAACAAT ATTCACTCT AATTAGACAT TTACTAAACT TTCTCTTGTGAA 8740  
 50 AACAAATGCCA AAAAAAGAAC ATTAGAAGAC ACGTAAGCTC AGTTGGTCTC TGCCACTAAG 8800  
 ACCAGCCAAC AGAAGCTTGA TTGTTATTCAA ACTTTGCAATT TTAGCATATT TTATCTTGTGAA 8860  
 AAATTCAATT GTGTTGGTTT TTGTTTTTG TTGTTATTGA ATAGACTCTC AGAAATCCAA 8920

55

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5	TTGTTGAGTA AATCTTCTGG GTTTCTAAC CTTTCTTAG AT ATT GAC CTC TGT Asp Ile Asp Leu Cys 255	8974
10	GAA AAC AGC GTG CAG CGG CAC ATT GGA CAT GCT AAC CTC ACC TTC GAG Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr Phe Glu 260 265 270	9022
15	CAG CTT CGT AGC TTG ATG GAA AGC TTA CCG GGA AAG AAA GTG GGA GCA Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val Gly Ala 275 280 285	9070
20	GAA GAC ATT GAA AAA ACA ATA AAG GCA TGC AAA CCC AGT GAC CAG ATC Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile 290 295 300	9118
25	CTG AAG CTG CTC AGT TTG TGG CGA ATA AAA AAT GGC GAC CAA GAC ACC Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr 305 310 315 320	9166
30	TTG AAG GGC CTA ATG CAC GCA CTA AAG CAC TCA AAG ACG TAC CAC TTT Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr His Phe 325 330 335	9214
35	CCC AAA ACT GTC ACT CAG AGT CTA AAG AAG ACC ATC AGG TTC CTT CAC Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His 340 345 350	9262
40	AGC TTC ACA ATG TAC AAA TTG TAT CAG AAG TTA TTT TTA GAA ATG ATA Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile 355 360 365	9310
45	GGT AAC CAG GTC CAA TCA GTA AAA ATA AGC TGC TTA TAACTGGAAA Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu 370 375 380	9356
50		

5	TGGCCATTGA GCTGTTCTT CACAATTGGC GAGATCCCCTT GGATGAGTAA ACTGTTCTC	9416
	AGGCCATTGA GGCTTTCAGT GATATCTTTC TCATTACCAAG TGACTAATT TGCCACAGGG	9476
	TACTAAAAGA AACTATGATG TGGAGAAAGG ACTAACATCT CCTCCAATAA ACCCCAAATG	9536
	GTAAATCCAA CTGTCAGATC TGGATCGTTA TCTACTGACT ATATTTCCC TTATTACTGC	9596
10	TTGCAGTAAT TCAACTGGAA ATTAAAAAAA AAAAAGTAGA CTCCACTGGG CCTTACTAAA	9656
	TATGGGAATG TCTAACCTAA ATAGCTTGG GATTCCAGCT ATGCTAGAGG CTTTATTAG	9716
	AAAGCCATAT TTTTTCTGT AAAAGTTACT AATATATCTG TAACACTATT ACAGTATTGC	9776
	TATTTATATT CATTAGATA TAAGATTTGG ACATATTATC ATCCTATAAA GAAACGGTAT	9836
15	GACTTAATT TAGAAAGAAA ATTATATTCT GTTTATTATG ACAAAATGAAA GAGAAAATAT	9896
	ATATTTTAA TGGAAAGTTT GTAGCATTCT TCTAACCTAGT ACTGCCATAT TTTCTGTGT	9956
	GGAGTATTTT TATAATTATA TCTGTATAAG CTGTAATATC ATTTTATAGA AAATGCATTA	10016
20	TTTAGTCAAT TGTTTAATGT TGGAAAACAT ATGAAATATA AATTATCTGA ATATTAGATG	10076
	CTCTGAGAAA TTGAATGTAC CTTATTAAA AGATTTATG GTTTATAAC TATATAATG	10136
	ACATTATTAA AGTTTCAA TTATTTTTA TTGCTTCTC TGTTGCTTT ATT	10190

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### Claims

30 1. A protein characterized by the following properties:

- (a) molecular weights on SDS-polyacrylamide gel electrophoresis (SDS-PAGE)
  - ; approximately 60 kD under reducing conditions
  - ; approximately 60 kD and 120 kD under non-reducing conditions
- (b) a high affinity to cation-exchange column and heparin column
- (c) a biological activity to inhibit osteoclast differentiation and/or maturation

40 : its activity is decreased by heating at 70°C for 10 min or at 56°C for 30 min.

: its activity is lost by heating at 90 °C for 10 min

(d) internal amino acid sequences provided in sequence numbers 1, 2, and 3.

45 2. A protein of claim 1 having N-terminal amino acid sequences provided in sequence number 7.

3. A protein of claim 1 produced in human fibroblasts.

4. A method of producing the protein of claim 1, 2, and 3 by the following process: cultivating human fibroblasts ; purifying the protein by a combination of ion-exchange column, affinity-column and reverse phase-column chromatography.

50 5. A method of producing the protein of claim 4 by cultivating human fibroblasts on alumina ceramic pieces.

55 6. A protein with amino acid sequence provided in sequence number 4.

7. cDNAs encoding amino acid sequence provided in sequence number 4.

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8. cDNA with nucleotide sequence provided in sequence number 6.
9. cDNAs that hybridize to cDNA provided in sequence number 6 under moderately stringent conditions.
- 5 10. A protein expressed from cDNA encoding amino acid sequence provided in sequence number 4.
11. A protein with a biological activity to inhibit osteoclast differentiation and/or maturation, that obtain as amino acid expressed cDNA sharing at least 80 % sequence identity with the amino acid sequence provided in sequence number 4.
- 10 12. A method of production of the protein with the following properties and inhibit osteoclast differentiation and/or maturation by gene engineering using cDNA encoding amino acid sequence provided in sequence number 4:
  - (a) molecular weights on SDS-polyacrylamide gel electrophoresis (SDS-PAGE)
    - 15 : approximately 60 kD under reducing conditions
    - : approximately 60 kD and 120 kD under non-reducing conditions
  - (b) a high affinity to cation-exchange column and heparin column
  - (c) : inhibit osteoclast differentiation and/or maturation activity is decreased by heating at 70°C for 10 min or at 56°C for 30 min
    - 20 ; its activity is lost by heating at 90 °C for 10 min
  - (d) internal amino acid sequence provided in sequence number 1-3.
13. A method of producing the protein according to claim 10 by gene engineering using mammalian cells as host cells.
14. A method of producing the protein according to claim 13 by gene engineering using 293/EBNA cells or CHO cells as mammalian host cells.
- 30 15. A cDNA with nucleotide sequence provided in sequence number 8.
16. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 8.
- 35 17. cDNAs encoding amino acid sequence provided in sequence number 9.
18. A cDNA with nucleotide sequence provided in sequence number 10.
- 40 19. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 10.
20. cDNAs encoding amino acid sequence provided in sequence number 11.
21. A cDNA with nucleotide sequence provided in sequence number 12.
- 45 22. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 12.
23. cDNAs encoding amino acid sequence provided in sequence number 13.
- 50 24. A cDNA with nucleotide sequence provided in sequence number 14.
25. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 14.
26. cDNAs encoding amino acid sequence provided in sequence number 15.
- 55 27. A cDNA with nucleotide sequence provided in sequence number 83.
28. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 83.

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29. cDNAs encoding amino acid sequence provided in sequence number 62.
30. A cDNA with nucleotide sequence provided in sequence number 84.
- 5 31. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 84.
32. cDNAs encoding amino acid sequence provided in sequence number 63.
- 10 33. A cDNA with nucleotide sequence provided in sequence number 85.
34. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 85.
35. cDNAs encoding amino acid sequence provided in sequence number 64.
- 15 36. A cDNA with nucleotide sequence provided in sequence number 86.
37. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 86.
38. cDNAs encoding amino acid sequence provided in sequence number 65.
- 20 39. A cDNA with nucleotide sequence provided in sequence number 87.
40. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 87.
- 25 41. cDNAs encoding amino acid sequence provided in sequence number 66.
42. A cDNA with nucleotide sequence provided in sequence number 88.
43. A protein encoded by a cDNA having a sequence provided in sequence number 88.
- 30 44. cDNAs encoding amino acid sequence provided in sequence number 67.
45. A cDNA with nucleotide sequence provided in sequence number 89.
- 35 46. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 89.
47. cDNAs encoding amino acid sequence provided in sequence number 68.
48. A cDNA with nucleotide sequence provided in sequence number 90.
- 40 49. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 90.
- 50 50. cDNAs encoding amino acid sequence provided in sequence number 69.
- 45 51. A cDNA with nucleotide sequence provided in sequence number 91.
52. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 91.
53. cDNAs encoding amino acid sequence provided in sequence number 70.
- 50 54. A cDNA with nucleotide sequence provided in sequence number 92.
55. A protein encoded by a cDNA having a nucleotide sequence provided in number 92.
- 55 56. cDNAs encoding amino acid sequence provided in sequence number 71.
57. A cDNA with nucleotide sequence provided in sequence number 93.

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58. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 93.
59. cDNAs encoding amino acid sequence provided in sequence number 72.
- 5 60. A cDNA with nucleotide sequence provided in sequence number 94.
61. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 94.
62. cDNAs encoding amino acid sequence provided in sequence number 73.
- 10 63. A cDNA with nucleotide sequence provided in sequence number 95.
64. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 95.
- 15 65. cDNAs encoding amino acid sequence provided in sequence number 74.
66. A cDNA with nucleotide sequence provided in sequence number 96.
67. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 96.
- 20 68. cDNAs encoding amino acid sequence provided in sequence number 75.
69. A cDNA with nucleotide sequence provided in sequence number 97.
- 25 70. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 97.
71. cDNAs encoding amino acid sequence provided in sequence number 76.
72. A cDNA with nucleotide sequence provided in sequence number 98.
- 30 73. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 98.
74. cDNAs encoding amino acid sequence provided in sequence number 77.
- 35 75. A cDNA with nucleotide sequence provided in sequence number 99.
76. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 99.
77. cDNAs encoding amino acid sequence provided in sequence number 78.
- 40 78. A cDNA with nucleotide sequence provided in sequence number 100.
79. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 100.
- 45 80. cDNAs encoding amino acid sequence provided in sequence number 79.
81. A cDNA with nucleotide sequence provided in sequence number 101.
82. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 101.
- 50 83. cDNAs encoding amino acid sequence provided in sequence number 80.
84. A cDNA with nucleotide sequence provided in sequence number 102.
- 55 85. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 102.
86. cDNAs encoding amino acid sequence provided in sequence number 81.

87. A cDNA with nucleotide sequence provided in sequence number 103.
88. A protein encoded by a cDNA having a nucleotide sequence provided in sequence number 103.
- 5 89. cDNAs encoding amino acid sequence provided in sequence number 82.
90. Genomic DNAs encoding the amino acid sequence provided in sequence number 4.
- 10 91. Genomic DNAs of Claim 90 with the nucleotide sequence provided in sequence number 104 or 105.
92. An antibody having specific affinity to the OCIF
- 15 93. An antibody of Claim 92 that is polyclonal antibody.
94. An antibody of Claim 92 that is monoclonal antibody.
- 20 95. A monoclonal antibody of Claim 94 being characterized by the following properties.  
Molecular weight of about 150,000, and of subclass IgG<sub>1</sub>, IgG<sub>2a</sub>, or IgG<sub>2b</sub>.
96. A method of determining the concentration of the protein of the OCIF using the antibodies of Claim 92, 93, 94, and 95.

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50

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Fig. 1

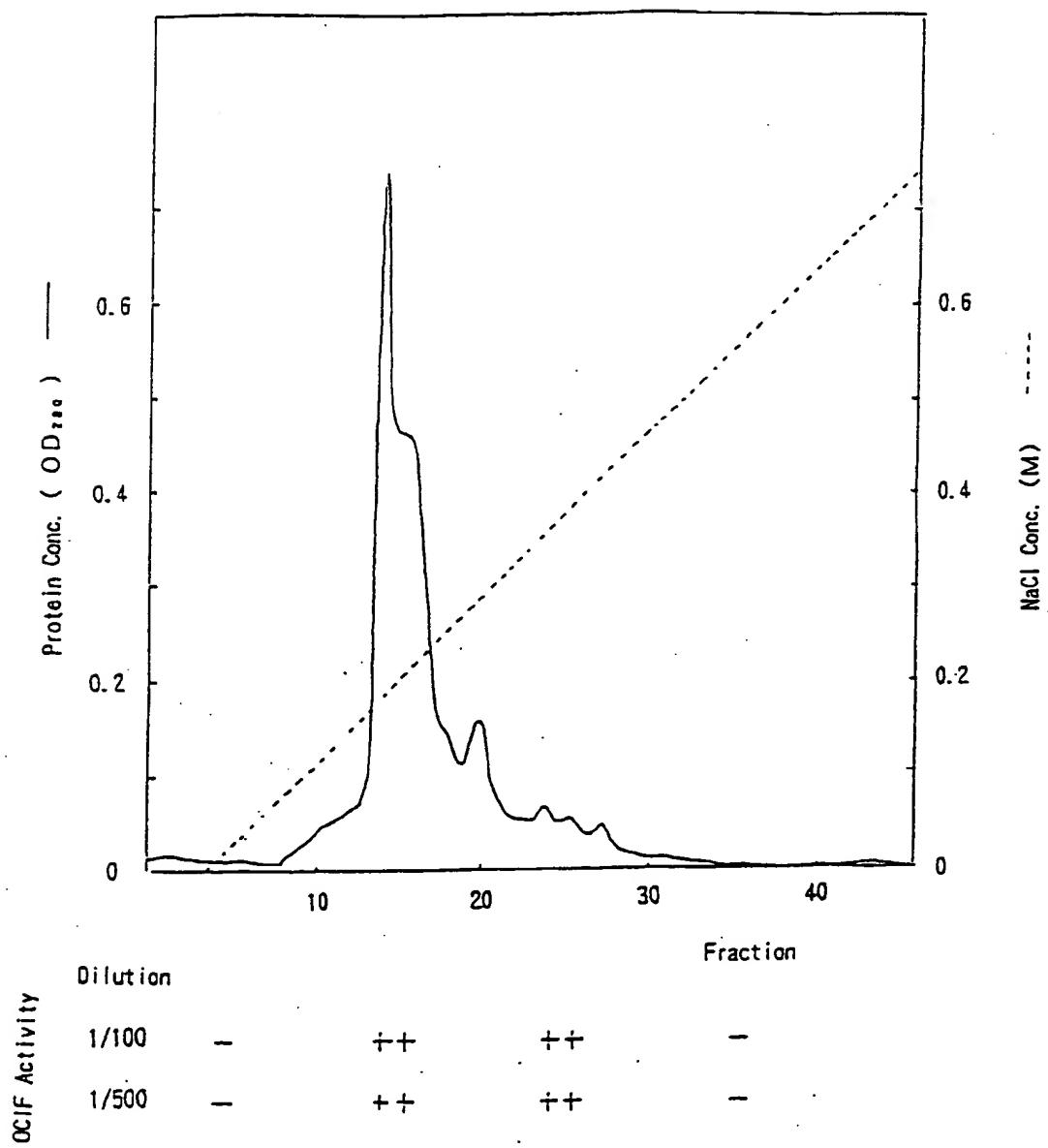


Fig. 2

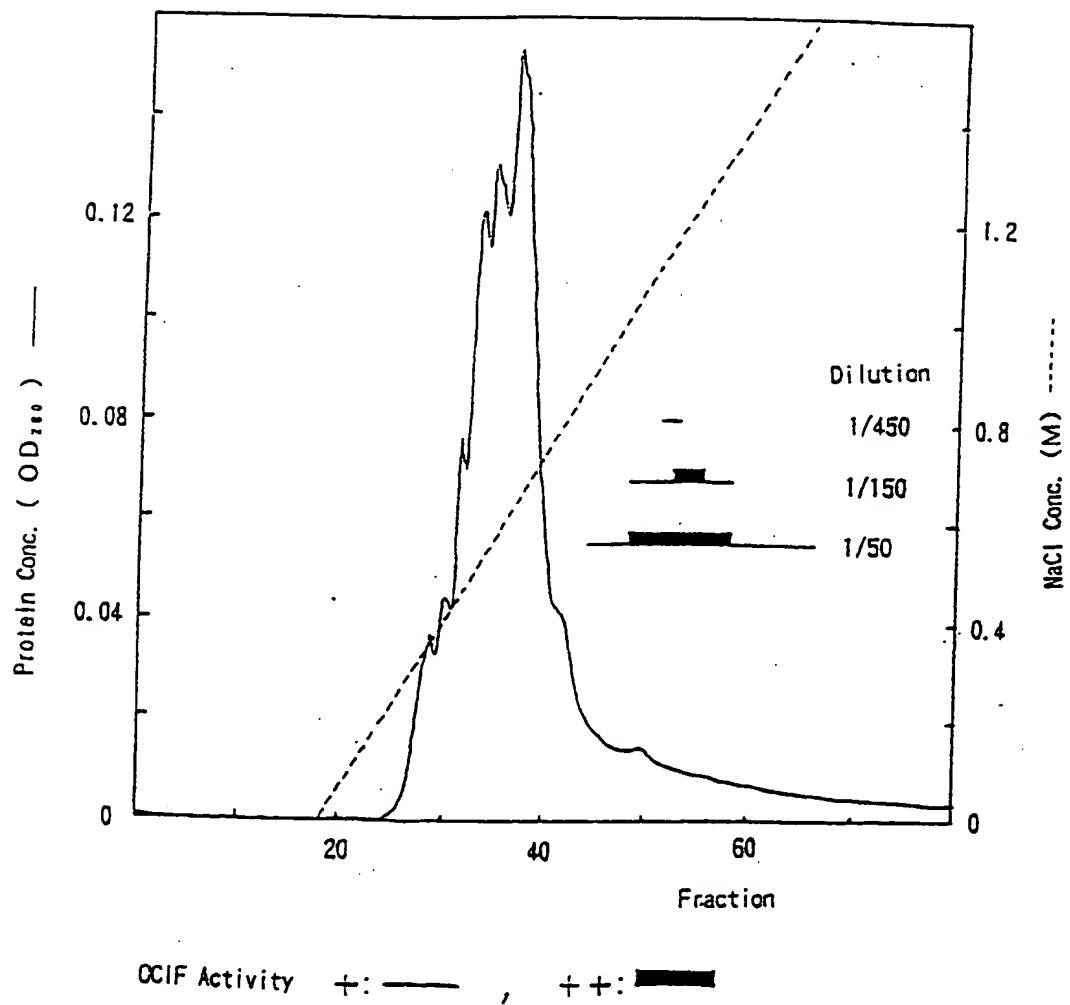


Fig. 3

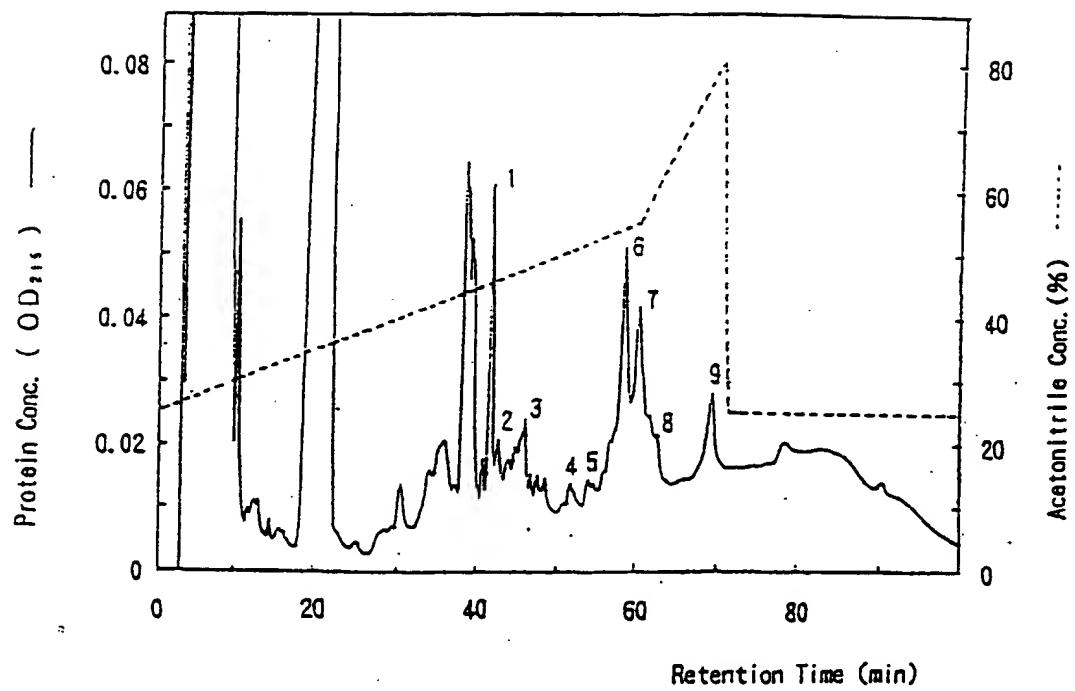


Fig. 4

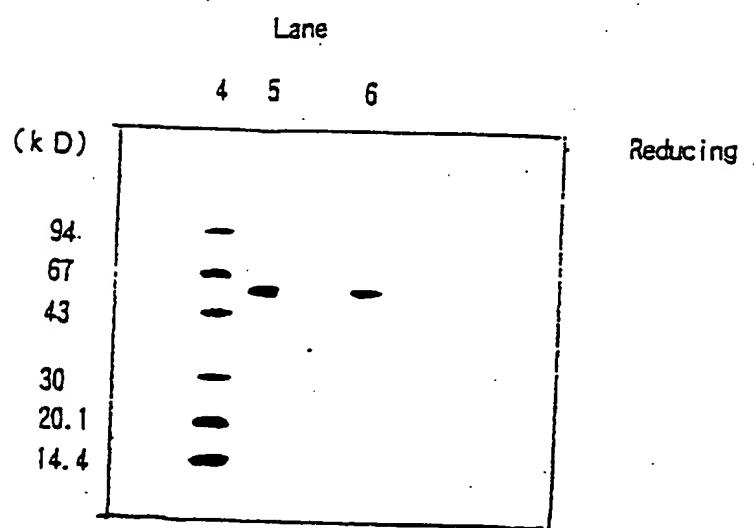
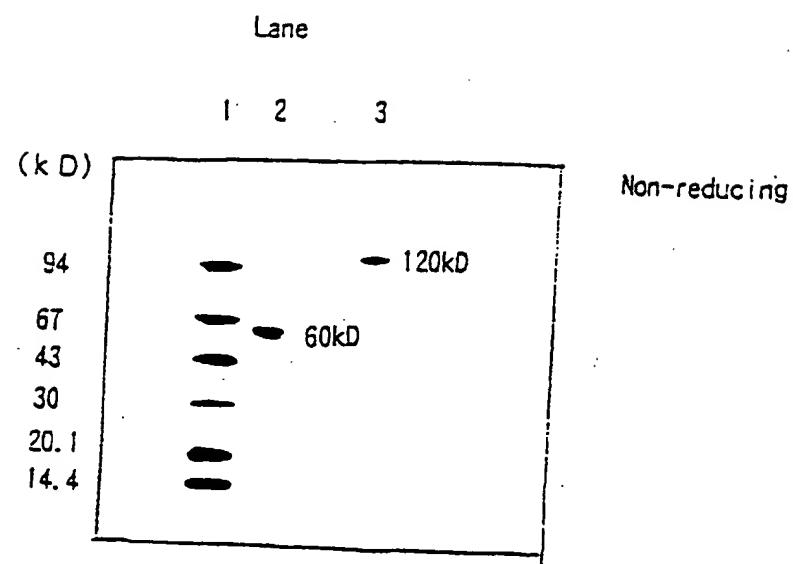
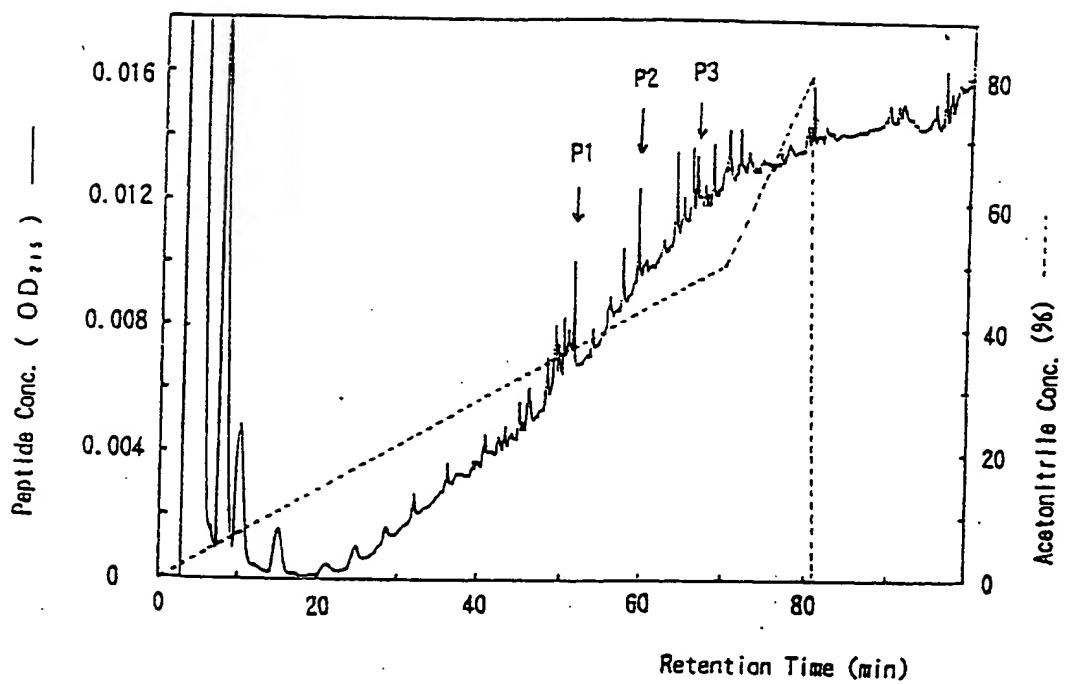


Fig. 5



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Fig. 6

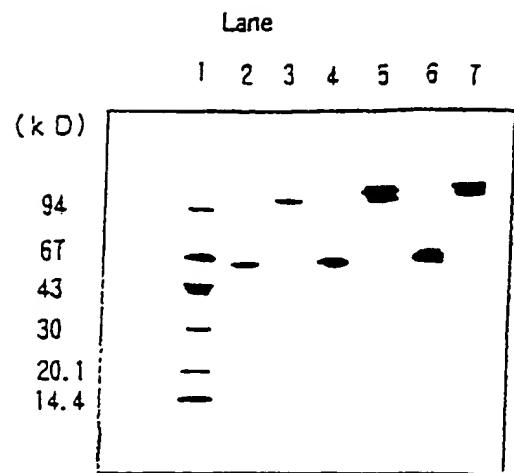


Fig. 7

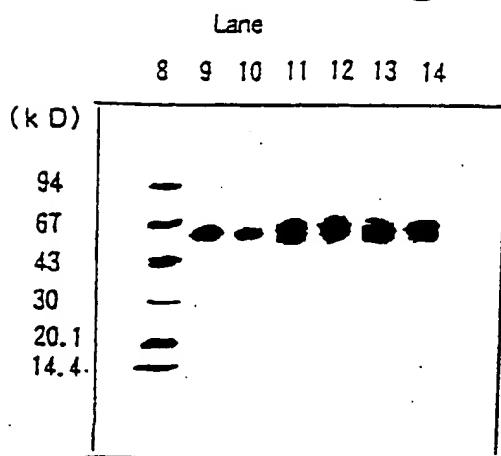


Fig. 8

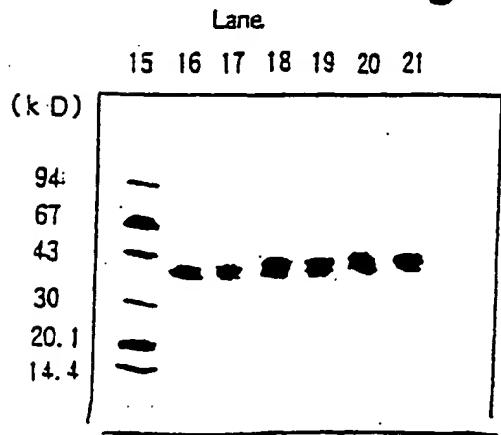


Fig. 9

1  
MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
\*\*\*\*\*  
MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF2)  
1

61  
VCAPCPDHYYTDSWHTSDECLYCSPPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
\*\*\*\*\*  
VCAPCPDHYYTDSWHTSDECLYCSPPVCKE-----CNRTHNRVCECKEGRYLEIEFCLK (OCIF2)  
61

121  
HRSCPPGFGVVQAGTPERNTVKRCPDGFFSNETSSKAPCRKHTNCVFGLLLQKGNAT (OCIF1)  
\*\*\*\*\*  
HRSCPPGFGVVQAGTPERNTVKRCPDGFFSNETSSKAPCRKHTNCVFGLLLQKGNAT (OCIF2)  
114

181  
HDNCSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF1)  
\*\*\*\*\*  
HDNCSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF2)  
174

241  
KRQHSSQEQTFLQLLKLWKHQNKDQDIVKKIIQDIDLCENSQQRHIGHANLTFEQLRSLME (OCIF1)  
\*\*\*\*\*  
KRQHSSQEQTFLQLLKLWKHQNKDQDIVKKIIQDIDLCENSQQRHIGHANLTFEQLRSLME (OCIF2)  
234

301  
SLPGKKVGAEDIETIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF1)  
\*\*\*\*\*  
SLPGKKVGAEDIETIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF2)  
294

361  
VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)  
\*\*\*\*\*  
VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF2)  
354

Fig. 10

1  
MNNLLCCALVLOISIKWTTQETFPPIKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
\*\*\*\*\*  
MNKLLCCALVLOISIKWTTQETFPPIKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF3)  
1

61  
VCAPCPDHYYTDSWHTSDECLYCSPIVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
\*\*\*\*\*  
VCAPCPDHYYTDSWHTSDECLYCSPIVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF3)  
61

121  
HRSCPPGFGVVQAGTPERNTVCKRCPOGFFSNETSSKAPCRKHTNCVFGLLLTKGNAT (OCIF1)  
\*\*\*\*\*  
HRSCPPGFGVVQAGTPERNTVCKRCPOGFFSNETSSKAPCRKHTNCVFGLLLTKGNAT (OCIF3)  
121

181  
HDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF1)  
\*\*\*\*\*  
HDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPWNLSVLVDNLPGTKVNAESVERI (OCIF3)  
181

241  
KRQHSSQEQTQFQLLKLWKHQNKDQDIVKKIIQDIDLCENSQVRHIGHANLTQEQLRSLME (OCIF1)  
\*\*\*\*\*  
KRQHSSQEQTQFQLLKLWKHQNKDQDIVKKIIQDIDLCENSQVRHIGHANL----- (OCIF3)  
241

301  
SLPGKKVGAEDIETIKACKPSDQILKLLSLWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF1)  
\*\*\*\*\*  
-----LWRIKNGDQDTLKGLMHALKHSKTYHFPKT (OCIF3)  
292

361  
VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF1)  
\*\*\*\*\*  
VTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL (OCIF3)  
322

## Fig. 11

1  
MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
\*\*\*\*\*  
MNKLLCCSLVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF4)  
1  
  
61  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
\*\*\*\*\*  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF4)  
61  
  
121  
HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTKGNAT (OCIF1)  
\*\*\*\*\*  
HRSCPPGFGVVQAGTCQCAAKLIRIMQSIVVTV (OCIF4)  
121

## Fig. 12

1  
MNNLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF1)  
\*\*\*\*\*  
MNKLLCCALVFLDISIKWTTQETFPPKYLHYDEETSHQLLCOKCPPGTYLKQHCTAKWKT (OCIF5)  
1  
  
61  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF1)  
\*\*\*\*\*  
VCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRVCECKEGRYLEIEFCLK (OCIF5)  
61  
  
121  
HRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHTNCSVFGLLLTKGNAT (OCIF1)  
\*\*\*\*\*  
HRSCPPGFGVVQAGCRRRPKPQICI (OCIF5)  
121

Fig. 13

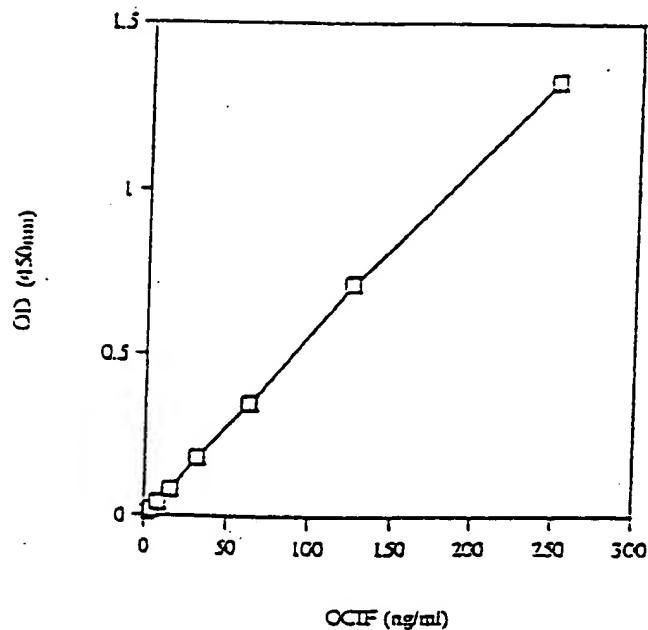


Fig. 14

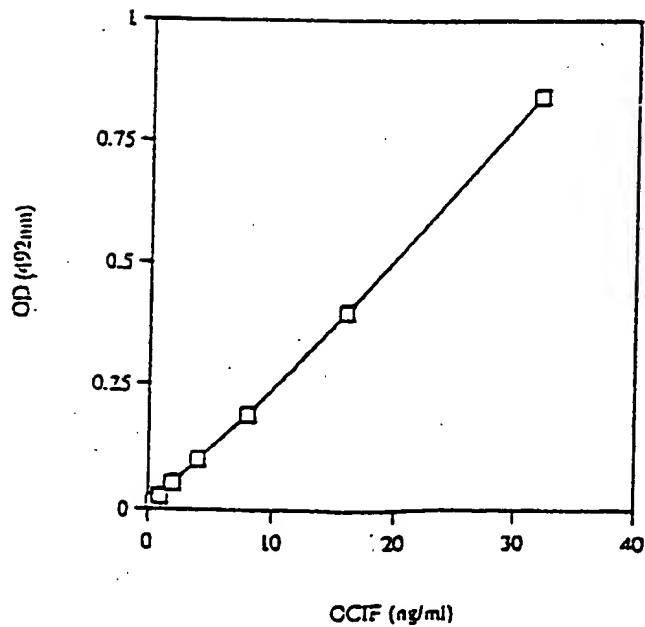
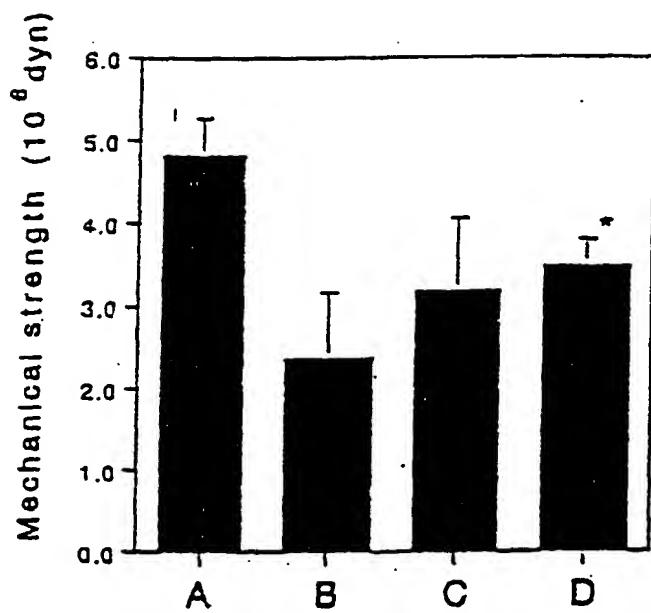


Fig. 15



- A : Normal rat
- B : Denervated rat + Vehicle
- C : Denervated rat + OCIF 10 $\mu$ g/kg/day
- C : Denervated rat + OCIF 100 $\mu$ g/kg/day

INTERNATIONAL SEARCH REPORT		International application No. PCT/JP96/00374									
<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl <sup>6</sup> C07K14/52, C07K16/24, C12N15/19, C12N15/06, C12N5/08, C12N5/10, C12N5/20, C12P21/02, C12P21/08, G01N33/577 According to International Patent Classification (IPC) or to both national classification and IPC											
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) Int. Cl <sup>6</sup> C07K14/52, C07K16/24, C12N15/19, C12N15/06, C12N5/08, C12N5/10, C12N5/20, C12P21/02, C12P21/08, G01N33/577											
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched											
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) BIOSIS PREVIEWS, CAS ONLINE, WPI, WPI/L											
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; padding: 2px;">Category*</th> <th style="text-align: left; padding: 2px;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="text-align: left; padding: 2px;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; padding: 2px;">A</td> <td style="padding: 2px;">Fawthrop, F.W. et al. "The effect of transforming growth factor beta on the plasminogen activator activity of normal human osteoblast-like cells and a human osteosarcoma cell line MG-63", J. Bone Miner. Res. (1992) Vol. 7, No. 12, p. 1363-1371</td> <td style="text-align: center; padding: 2px;">1 - 96</td> </tr> <tr> <td style="text-align: center; padding: 2px;">A</td> <td style="padding: 2px;">Fenton, A.J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. (1993) Vol. 155, No. 1, p. 1-7</td> <td style="text-align: center; padding: 2px;">1 - 96</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	A	Fawthrop, F.W. et al. "The effect of transforming growth factor beta on the plasminogen activator activity of normal human osteoblast-like cells and a human osteosarcoma cell line MG-63", J. Bone Miner. Res. (1992) Vol. 7, No. 12, p. 1363-1371	1 - 96	A	Fenton, A.J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. (1993) Vol. 155, No. 1, p. 1-7	1 - 96
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A	Fenton, A.J. et al. "Long-term culture of disaggregated rat osteoclasts inhibition of bone resorption and reduction of osteoclast-like cell number by calcitonin and PTHrP107-139", J. Cell Physiol. (1993) Vol. 155, No. 1, p. 1-7	1 - 96									
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.											
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed											
Date of the actual completion of the international search May 14, 1996 (14. 05. 96)		Date of mailing of the international search report May 28, 1996 (28. 05. 96)									
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer  Telephone No.									

binant plasmid containing the 5.8 kb EcoRI/NotI fragment was isolated and this plasmid was termed pBSG8-5.8. pBSG8-5.8 was digested with HindIII and 0.9 kb of DNA fragment derived from this digestion was isolated in the same manner as described above. This 0.9 kb fragment was then cloned in pBluescript II SK- at the HindIII site as described above. This recombinant plasmid containing 0.9 kb HindIII fragment was denoted pBS8H0.9.

6       $\lambda$ OIF11 DNA was digested with EcoRI and 6 kb, 3.6 kb, 2.6 kb EcoRI fragments were isolated in the same manner as described above and cloned in pBluescript II SK+ vector at the EcoRI site as described above. These recombinant plasmids were termed pBSG11-6, pBSG11-3.6, and pBSG11-2.6, respectively. pBSG11-6 was digested with HindIII and the digest was applied on a 0.7 % agarose gel. Three fragments, 2.2 kb, 1.1 kb, and 1.05 kb in length, were extracted from the gel and cloned independently in pBluescript II SK- vector at the HindIII site in the same manner as 10 described above. These recombinant plasmids were termed pBS6H2.2, pBS6 H1.1 and pBS6H1.05, respectively.

15      The nucleotide sequence of the cloned genomic DNA was determined using ABI DyeDeoxy Terminator Cycle Sequencing Ready Reaction Kit (PERKIN ELMER) and 373A DNA Sequencing system (Applied Biosystems). Plasmids pBSG8-5.8, pBS8H0.9, pBSG11-6, pBSG11-3.6, pBSG11-2.6, pBS6H2.2, pBS6H1.1 and pBS6H1.05 were prepared according to the alkaline-SDS procedure as described in Molecular Cloning: A Laboratory Manual and used as 15 templates for the DNA sequence analysis. Nucleotide sequence of the human OCIF gene was presented in Sequence No 104 and Sequence No 105. The nucleotide sequence of the DNA, between exon 1 and exon 2 was not entirely determined. There is a stretch of approximately 17 kb of nucleotides between the sequences given in sequence No. 104 and sequence No. 105.

20      EXAMPLE 24

Quantitation of OCIF by EIA

i) Preparation of anti-OCIF antibody

25      Male JW rabbits (Kitayama LABES Co., LTD) weighing 2.5-3.0 kg were used for immunization for preparing antisera. Three male JW rabbits (Kitayama LABES Co., LTD) weighing 2.5-3.0 kg were used for immunization. For immunization, emulsion was prepared by mixing an equal volume of rOCIF (200  $\mu$ g/ml) and complete Freund's adjuvant (Difco, Cat. 0638-60-7). The rabbits were immunized subcutaneously six times at the interval of one week with 1 ml of emulsion 30 per injection. The rabbits were injected six times at the interval of seven days subcutaneously. Whole blood was obtained ten days after the final immunization and serum was separated. Antibody was purified from serum as follows. Antiserum was diluted two-fold with PBS. After adding ammonium sulfate at a final concentration of 40 w/v %, antiserum was allowed to stand at 4 °C for 1 hr.. Precipitate obtained by centrifugation at 8000 x g for 20 min. was dissolved in a small volume of PBS and was dialyzed against PBS. The resulting solution was loaded onto a Protein G-Sepharose column (Pharmacia). After washing with PBS, absorbed immunoglobulin G was eluted with 0.1 M glycine-HCL buffer (pH 3.0). Elutes were neutralized with 1.5 M Tris-HCL buffer (pH 8.7) immediately and were dialyzed against PBS. Protein concentration was determined by absorbance at 280nm (E<sup>1%</sup> 13.5).

35      Horseradish peroxidase labeled antibody was prepared using ImmunoPure Maleimide Activated Horseradish Peroxidase Kit (Pierce, Cat. 31494). Briefly, one mg of IgG was incubated with 80  $\mu$ g of N-succinimidyl-S-acetylthioacetate for 30 min. After deacetylation with 5 mg of hydroxylamine HCl, modified IgG was separated by polyacrylamide desalting column. Protein pool mixed with one mg of maleimide activated horseradish peroxidase was incubated at room temperature for 1 hr.

40      ii) Quantitation of OCIF by sandwich EIA

45      Microtiter plates (Nunc MaxiSorp Immunoplate) were coated with rabbit anti-OCIF IgG by incubating 0.2  $\mu$ g in 100  $\mu$ l of 50 mM sodium bicarbonate buffer pH 9.6 at 4C overnight. After blocking the plates by incubating for 1 hour at 37°C with 300  $\mu$ l of 25% BlockAce/PBS (Snow Brand Milk Products ), 100 $\mu$ l of samples were incubated for 2 hours at room temperature. After washing the plates three times with PBST (PBS containing 0.05% Tween20), 100  $\mu$ l of 1:10000 50 diluted horseradish peroxidase labeled anti-OCIF IgG was added and incubated for 2 hours at room temperture. The amount of OCIF was determined by incubation with 100  $\mu$ l of a substrate solution (TMB, ScyTek Lab., Cat. TM4999) and measurement of the absorbance at 450 nm using an ImmunoReader (Nunc NJ2000). Purified recombinant OCIF was used as a standard protein and a typical standard curve was shown in Fig. 13.

## EXAMPLE 25

## Anti-OCIF monoclonal antibody

## 5 i) Preparation of hybridoma producing anti-OCIF monoclonal antibody.

OCIF was purified to homogeneity from culture medium of human fibroblasts, IMR-90 by the purification method described in Example 11. Purified OCIF was dissolved in PBS at a concentration of 10 µg/100 µl. BALB/c mice were immunized by administrating this solution intraperitoneally three times every two weeks. In the first and the second 10 immunizations, the emulsion composed of an equal volume of OCIF and Freund's complete adjuvant was administered. Three days after the final administration, the spleen was taken out, lymphocytes were isolated and fused with mouse myeloma p3x63-Ag8.653 cells according to the conventional method using polyethyleneglycol. Then the fused cells were cultured in HAT medium to select hybridoma. Subsequently, to check whether the selected hybridomas produce anti-OCIF antibody, anti-OCIF antibody in each culture medium of hybridomas was determined by solid phase ELISA which 15 was prepared by coating each well in 96-well immunoplates (Nunc) with 100µl of purified OCIF (10µg/ml in 0.1 M NaHCO<sub>3</sub>) and by blocking each well with 50% BlockAce (Snow Brand Milk Products Co. Ltd.). The hybridoma clones secreting anti-OCIF antibody were established by cloning 3 - 5 times by limit dilution and by screening using the above solid phase ELISA. Among thus obtained hybridoma clones, several hybridoma clones with high production of anti-OCIF antibody were selected.

## 20 ii) Production of anti-OCIF monoclonal antibodies.

Each hybridoma clone secreting anti-OCIF antibody, which was obtained in EXAMPLE 25-i), was transplanted intraperitoneally to mice given Pristane (Aldrich) at a cell density of 1 x 10<sup>6</sup> cells/mouse. The accumulated ascites was collected 25 10 - 14 days after the transplantation and the ascites containing anti-OCIF specific monoclonal antibody of the present invention was obtained. Purified antibodies were obtained by Affigel protein A Sepharose chromatography (BioRad) according to the manufacturer's manual. That is, the ascites was diluted with equal volume of a binding buffer (BioRad) and applied to protein A column. The column was washed with a sufficient volume of the binding buffer and eluted with an elution buffer (BioRad). After neutralizing, the obtained eluate was dialyzed in water and subsequently lyophilized. 30 The purity of the obtained antibody was analyzed by SDS/PAGE and a homogenous band with a molecular weight of about 150,000 was detected.

## iii) Selection of monoclonal antibody having high affinity to OCIF

35 Each antibody obtained in EXAMPLE 25-ii) was dissolved in PBS and the concentration of protein in the solution was determined by the method of Lowry. Each antibody solution with the same concentration was prepared and then serially diluted with PBS. Monoclonal antibodies, which can recognize OCIF even at highly diluted solution, were selected by solid phase ELISA described in EXAMPLE 25-ii). Thus three monoclonal antibodies A1G5, E3H8 and D2F4 can be selected.

## 40 iv) Determination of class and subclass of antibodies

The class and subclass of the antibodies of the present invention obtained in EXAMPLE 25-iii) were analyzed using an immunoglobulin class and subclass analysis kit (Amersham). The procedure was carried out according to the protocol disclosed in the directions. The results were shown in Table 15. The antibodies of the present invention, E3H8, A1G5 and D2F4 belong to IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub>, respectively.

Table 15

Analysis of class and subclass of the antibodies in the present invention.							
Antibody	IgG <sub>1</sub>	IgG <sub>2a</sub>	IgG <sub>2b</sub>	IgG <sub>3</sub>	IgA	IgM	κ
A1G5	-	+	-	-	-	-	+
E3H8	+	-	-	-	-	-	+
D2F4	-	-	+	-	-	-	+

## v) Determination of OCIF by ELISA

5 Three kinds of monoclonal antibodies, A1G5, E3H8 and D2F4, which were obtained in EXAMPLE 25-iv), were used as solid phase antibodies and enzyme-labeled antibodies, respectively. Sandwich ELISA was constructed by each combination of solid phase antibody and labeled antibody. The labeled antibody was prepared using Immuno Pure Maleimide Activated Horseradish Peroxidase Kit (Pierce, Cat. No. 31494). Each monoclonal antibody was dissolved in 0.1 M NaHCO<sub>3</sub> at a concentration of 10 µg/ml, and 100 µl of the solution was added to each well in 96-well immunoplates (Nunc, MaxiSorp Cat. No. 442404) followed by allowing to stand at room temperature overnight. Subsequently, each well in the plates was blocked with 50% Blockace (Snow Brand Milk Products, Co. , Ltd. ) at room temperature for 10 50 minutes, and then was washed three times with PBS containing 0.1% Tween 20 (washing buffer).

10 A series of concentrations of OCIF was prepared by diluting OCIF with 1st reaction buffer (0.2 M Tris-HCl buffer, pH 7.4, containing 40% Blockace and 0.1% Tween 20). Each well in 96-well immunoplates was filled with 100 µl of the prepared OCIF solution with each concentration, allowed to stand at 37 °C for 3 hours, and subsequently washed three times with the washing buffer. For dilution of POD-labeled antibody, 2nd reaction buffer (0.1 M Tris-HCl buffer, pH 7.4, containing 25% Blockace and 0.1% Tween 20) was used. POD-labeled antibody was diluted 400-fold with 2nd reaction 15 buffer, and 100 µl of the diluted solution was added to each well in the immunoplates. Each immunoplate was allowed to stand at 37 °C for 2 hours, and subsequently washed three times with the washing buffer. After washing, 100 µl of a substrate solution (0.1 M citrate-phosphate buffer, pH 4. 5, containing 0.4 mg/ml of o-phenylenediamine HCl and 0.006% H<sub>2</sub>O<sub>2</sub>) was added to each well in the immunoplates and the immunoplates were incubated at 37°C for 15 min. 20 The enzyme reaction was terminated by adding 50 µl of 6 N H<sub>2</sub>SO<sub>4</sub> to each well. The optical density of each well was determined at 492 nm using an immunoreader (ImmunoReader NJ 2000, Nunc).

25 Using three kinds of monoclonal antibody in the present invention, each combination of solid phase and POD-labeled antibodies leads to a accurate determination of OCIF. Each monoclonal antibody in the present invention was confirmed to recognize a different epitope of OCIF. A typical standard curve of OCIF using a combination of solid phase antibody, A1G5 and POD-labeled antibody, E3H8 was shown in Fig. 14.

## vi) Determination of OCIF in human serum

30 Concentration of OCIF in five samples of normal human serum was determined using an EIA system described in EXAMPLE 25-v). The immunoplates were coated with A1G5 as described in EXAMPLE 25-v), and 50 µl of 1st. reaction buffer was added to each well in the immunoplates. Subsequently, 50 µl of each human serum was added to each well in the immunoplates. The immunoplates were incubated at 37°C for 3 hours and then washed three times with the washing buffer. After washing, each well in the immunoplates was filled with 100 µl of POD-E3H8 antibody diluted 400-fold with 2nd. reaction buffer and incubated at 37°C for 2 hours. After washing the immunoplates three times with the 35 washing buffer, 100 µl of the substrate solution described in EXAMPLE 25-v) was added to each well and incubated at 37°C for 15 min. The enzyme reaction was terminated by adding 50 µl of 6 N H<sub>2</sub>SO<sub>4</sub> to each well in the immunoplates. The optical density of each well was determined at 492 nm using an immunoreader (ImmunoReader NJ 2000, Nunc). 1st. reaction buffer containing the known amount of OCIF was treated in the same way and a standard curve of OCIF as shown in fig. 2 was obtained. Using the standard curve of OCIF, the amount of OCIF in human serum sample was 40 determined. The results were shown in Table 14.

Table 14

The amount of OCIF in normal human serum	
Serum Sample	OCIF Concentration (ng/ml)
1	5.0
2	2.0
3	1.0
4	3.0
5	1.5